ASSESSMENT OF PATHOGENIC BACTERIA AND HEAVY METAL POLLUTION IN SEDIMENT AND WATER OF KAHWA RIVER, BUKAVU, DEMOCRATIC REPUBLIC OF THE CONGO

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Declaration

I declare that ASSESSMENT OF PATHOGENIC BACTERIA AND HEAVY METAL POLLUTION IN SEDIMENT AND WATER OF KAHWA RIVER, BUKAVU, DEMOCRATIC REPUBLIC OF THE CONGO is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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Abstract

Anthropogenic activities generate waste products that pollute the environment with bacteria and heavy metals. This research assessed pollution of the Kahwa River, Bukavu Town, DRC with cadmium and lead (HMs) and bacterial enteropathogens. A survey of businesses, households and healthcare facilities showed general use of the river to remove effluent and waste. Indicator organisms were cultured at over 200 cfu/100 ml showing faecal contamination of the river water. Antibiotic resistance was shown by enteropathogenic *Vibrio cholerae* and *Salmonella typhi* to ampicillin and cotrimoxazole with some sensitivity shown to ciprofloxacin. River water contained HMs at around 40 times the World Health Organisation limit for drinking water. The bacteria, particularly from river sediment, tolerated HMs up to a concentration of 1.5 mg/ml. The presence in the Kahwa River of antibiotic-resistant pathogens showing tolerance to HMs has serious public health implications.

Key words

Cadmium, Lead, pathogenic bacteria, pollution, antibiotics, antibiotic resistance bacteria, heavy metal resistance bacteria, indicator bacteria, Kahwa River, Bukavu Town, Kahwa River catchment, water, sediment, Democratic Republic of the Congo.

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Abbreviations

AAS:	Atomic Absorption Spectrometer
Amp:	Ampicillin
APW:	Alkaline Peptone Water
ARB:	Antibiotic resistance bacteria
ARG:	Antibiotic resistance genes
BHIB:	Brain Heart Infusion Broth
BPF:	Binding Protein Factor
BPW:	Buffered Peptone Water
C:	Chloramphenicol
CBA:	Cassette Binding ATP transporters
Cd:	Cadmium
CDF:	Cation Diffusion Facilitator transporters
Cip:	Ciprofloxacin
CLSI:	Clinical and Laboratory Standard Institute
CRB:	Cadmium Resistance Bacteria
DDM:	Disk Diffusion Method
E. coli:	*Escherischia coli*
EPA:	Environment Protection Agency
HIA:	Heart Infusion Agar
HMRB:	Heavy metal resistance bacteria
HMRG:	Heavy metal resistance genes
HMs:	heavy metals
HMTA:	Heavy Metal transporters ATPase
KIA:	Kligler Iron Agar
MBDs:	MetalBinding Domains
MFP:	Membrane Fusion Protein
MH:	Mueller Hinton
OMF:	Outer Membrane Factor
Pb:	Lead
PRB:	Lead resistance bacteria

PVC:	Polyvinyl Chloride Plastics
RMD:	Resistance Modulation and cell Division super family
S. dysenteriae:	*Shigella dysenteriae*
S. paratyphi:	*Salmonella paratyphi*
S. typhi:	*Salmonella typhi*
Sxt:	Cotrimaxazole
TCBS:	Thiosulfate Citrate Bile Salt
Tet:	Tetracycline
TSI:	Triple Iron agar
TTC:	Tetrazolium Chloride
UNEP:	United Nations for Environmental Protection Agency
V. cholerae:	*Vibrio cholerae*
WHO:	World Health Organization
XLD:	Xylose Lysine Desoxycholate

Dedication

I thank my mother who supported and showed love and interest in my studies. She has been killed innocently by wicked people without seeing this great achievement in 2012. May God grant your soul to rest in peace forever.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

Environmental pollution is a major problem worldwide particularly that involving heavy metals (HMs) and pathogenic bacteria (Nageswaran *et al*., 2012 and Czekalski *et al*., 2012). There appears to be a microenvironmental link between HMs and bacteria as research has reported that HMs have been found at increasing levels within bacterial environments (Narasimhulu *et al*., 2010; Nageswaran *et al*., 2012). Metals like copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) are essential micronutrients in bacterial metabolism (Abdelatey *et al*., 2011; Nasrazadani *et al*., 2011) wher they are involved in redox processes and stabilize molecules through electrostatic interactions. In addition, they are co-factors in enzymatic reactions and regulate osmotic balance (Nies, 1999; Bruins *et al*., 2000; Adbelatey *et al*., 2011). Essential metals are also involved in the expression of genes and stabilize DNA structure (Śmejkalová, 2003; Egbenni *et al*, 2010; Intorne *et al*., 2012). However, a physiological role by HMs like Cadmium (Cd) and Lead (Pb) is not known as they are toxic to bacterial cells, even at low concentration (WHO, 2007).

The toxicity of these metals is linked to their capacity to interact with nucleic acids, to bind essential proteins and to displace essential metals from their natural binding sites in proteins and organic molecules (Hynnien, 2010). In addition, Cd and Pb can damage bacterial cell membranes, alter enzyme specificity properties, disrupt cellular functions and damage the structure of DNA (Nies, 1999). Inside the bacterial cell, the toxicity of Cd and Pb ions may occur through the displacement of essential metals from their native binding site or through ligand interactions, where they can modify the structure of essential proteins (Nies, 1999, Bruins *et al*., 2000). Furthermore, Cd and Pb can interact with physiological ions to then inhibit the physiological function of cations (Nies, 1999).

Thus, bacteria that take up HMs may experience an altered genome, proteome and/or metabalomic environment (Nies, 1999, Nageswaran *et al*., 2012) that may contribute significantly to altered bacterial metabolism and their

environments that may, in turn, impact negatively on human beings, plants and animals associated with such bacteria (Cubaka, 2010).

In order to survive in a metal-polluted aquatic environment, bacteria have evolved mechanisms to regulate metal uptake (Nasrazadani *et al*., 2011). These mechanisms include metal exclusion by permeability barriers, metals efflux, intracellular HMs sequestration by some proteins, extracellular sequestration and enzymatic detoxification to less toxic form, accumulation and complexation of the metals inside the cell and the oxidation and reduction of ions of HMs to a less toxic state (Nasrazadani *et al*., 2011; Skirumaran *et al*., 2011).

Recent research has shown a correlation between tolerance to HMs and resistance to antibiotics in bacteria (Spain and Alm, 2003; Nasrazadani *et al*., 2011). Resistance to antibiotics and tolerance to HMs involves resistance genes that bacteria may gain from other microbes through a variety of changes to its genome via conjugational, transductional and transformational processes and/or vertical gene transfer (Abdelatey *et al*., 2011). Antibiotic resistant bacteria (ARB) constitute another major global problem that has led to difficulties and raised costs of treating infectious diseases that may increase mortality and morbidity rates (Yah and Eghafona, 2008). Once acquired, resistance genes are not easily lost by microbes and these genes become a relatively stable part of the bacterial genome that is then transferred to the next generation (Czekalski *et al*., 2012).

In addition, ARB ingested in the gut may interact with the normal flora and possibly transfer resistance genes to these gut flora, that, in turn, may transfer resistance genes amongst themselves (Yah and Eghafona, 2008). Thus, the possible persistance and dissemination of ARB into an aquatic environment may contribute to an increase in infections involving resistant bacteria exacerbated by the transfer of antibiotic resistance into current and emerging pathogens (Mudryk, 2002; Czekalski *et al*., 2012).

The growing threat from resistant organisms, caused by increased HMs pollution into aquatic environment like the Kahwa River, should alert scientists and political authorities of the need for concerted action in order to prevent the emergence of new resistant strains and the spread of existing ARB. Although bacteria tolerant to HMs play a negative role in antibiotic resistance, they have

important environmental implications in the removal of heavy metals in contaminated aquatic ecosystems (Spain and Alm, 2003). Thus, they may be applied in agricultural lands to clean up toxic HMs and antibiotics from polluted cropland in order to promote plant growth by increasing the growth of essential soil microbes (rhizobacteria) (Van der Heijden *et al*., 2008; Saharan and Nehra, 2011).

The Kahwa River flowing through Bukavu Town in the Democratic Republic of the Congo is highly influenced by associated anthropogenic activities resulting in its pollution (Mubwebwe, 2009 and Bagalwa *et al*., 2013). Factors contributing to this pollution include factory activities and generated waste, increased population growth, uncontrolled disposal of animal and human waste, healthcare facilities and garages. In relation to this study, the waste from these activities may lead to pollution of river sediment and water with HMs particularly Cd and Pb, as well as pathogenic bacteria.

Most toilets in the Kahwa River catchment consist of pit latrines and families that do not have such facilities living along the river release faeces directly into it. This may result in the contamination of sediment and water with pathogenic bacteria (Lotter, 2010). In addition, Bukavu town is home to vehicles and motorbikes that use fossil-based fuels and oil. According to the WHO (2007), the traffic may result in the discharge of Cd and Pb into the environment. Moreover, most pipes distributing potable water throughout Bukavu town are made of lead and may increase Pb pollut in water (Blinda, 2005).

1.2. Problem Statement

The dual pollution of the Kahwa River with microbes and HMs may pose a health risk to human and aquatic life. Mutuku *et al*. (2014) reported that bacteria evolving in a Cd- and Pb-polluted aquatic environment may develop resistance mechanisms following their acquisition of resistance genes from other resistant microbes and/ via mutations. These mechanisms may contribute to the spread of antibiotic resistance among bacteria making up microbial biofilms in the environment (Czekalski *et al*., 2012).

Water of the Kahwa River is not consumed directly by the population because of its unappealing green-yellowish color resulting from soil erosion and

human waste disposal (Mubwebwe, 2009). However, it is used to wash cars and clothes (Bagalwa *et al*., 2013) before flowing into Lake Kivu whose water is directly consumed by the population. These inputs can contribute to an increase in HMs and bacteriological pollution in the sediment and water of the river and Lake Kivu. Recently, the population has increased in the Kahwa River catchment area due to migration from the rural areas, to search for jobs in the town and for personal security reasons. This has led to an increase in the pollution of the Kahwa River (Bagalwa *et al*., 2013).

The plant facility, REGIDESO, which treats and distributes potable water throughout the town, is currently unable to respond to population needs (Bisangano *et al*., 2012). This obliges the citizens to fetch water in Lake Kivu for domestic use. Thus, the lake becomes the source of water for families, for swimming and for other recreational activities such as fishing (Mubwebwe, 2009). Hence, the pollution of Kahwa River and, subsequently, Lake Kivu will not only damage aquatic life but also impact on human health. This, in turn, will impact on the national economy and can lead to increased costs required to expand water treatment facilities and to develop an alternative potable water source. It also contributes to degradation or loss of habitat and biodiversity and related loss in tourism revenue, direct and indirect costs of diseases including treatment costs and reduced economic productivity through increased morbidity and mortality.

Several research projects have been carried out on the Kahwa River and its catchment including (i) a calculation of land use/land cover around the Kahwa River from 1986 to 2010 (Bagalwa *et al*., 2014), (ii) an estimation of the pollutant load transported from Kahwa River micro-catchment into Lake Kivu (Bagalwa *et al*., 2013), (iii) a determination of bacteriological pollution of ground water sources used by local communities living in and around Bukavu Town (Bisangamo *et al*., 2012), (iv) an assessment of knowledge of the population of Bukavu Town (Ibanda district) as to management of domestic waste (Ntabugi, 2013), (v) a determination of macroinvertabrate diversity regarding arthropods, annelids, mollusks, platyhelminths and nematodes in the rivers of Bukavu Town, and (vi) an analysis of the impact of waste from anthropogenic activities on water quality and its management in Lake Kivu basin (Mubwebwe, 2009).

However, no research has been carried out to assess the pollution of sediment and water in the Kahwa River with HMs such as Cd and Pb, nor for pathogenic bacteria. Furthermore, no research has been performed in the Kahwa River catchment to study the source of HMs and bacteria pollution in this river. This study will help to develop baseline knowledge of water quality in the river in order to reduce the negative impacts associated with pollution of its sediment and water.

1.3. Research hypothesis

The waste discharged into the Kahwa River pollutes the sediment and water with pathogenic bacteria and HMs, particularly Cd and Pb, at levels above acceptable limits. Isolated bacteria are likely to be resistant to antibiotics and HMs.

1.4. Research Aims and Objectives

This research is aimed at determining the current water quality in the Kahwa River as to the types of pollution deposited into the Kahwa River and the impact this pollution has on contamination of the river with antibiotic-resistant and heavy metal-tolerant pathogenic bacteria. In achieving these aims, the following objectives were completed:

- To collect sediment and water samples from selected sites in the Kahwa River, Bukavu Town, Democratic Republic of the Congo
- To analyse these samples using atomic absorption spectrometry for the presense of heavy metals such as cadmium and lead
- To analyse these samples for the presense of indicator and gastroenteritis-associated bacteria
- To perform antibiotic and HMs susceptibility tests on bacterial isolates

1.5. Value and Benefits

The Kahwa River passes throughout Bukavu Town where it receives waste from anthropogenic activities which can result in pollution by HMs and bacteria of the river's sediment and water. To survive within a metal-polluted aquatic ecosystem, bacteria develop resistance mechanisms leading to the proliferation of

antibiotic- and HMs-resistant bacteria in the environment (Spain and Alm, 2003, Nasrazadani *et al*., 2010). Such resistant bacteria may be used to play a beneficial environmental role in the removal of metals in polluted areas (Spain and Alm, 2003). Meanwhile, ARB can increase the rate of morbidity and mortality in the biodiversity. Feedback of the research results must be provided to citizens of Bukavu Town and the research should be published in peer-reviewed journals to sensitize the population and public authorities in order to reduce the pollution of this river.

1.6. Dissertation outline

This dissertation is divided into five chapters:

The first is a general introduction. It consists of the background which explains the role played by anthropogenic waste in the pollution of sediment and water of an urban river, particularly with heavy metals and bacteria. It also explains the role of such pollution in the spread of antibiotic and heavy metal resistance in bacteria within the ecosystem and the problems that these resistant bacteria may cause. It concludes with the aims and the objectives of the research, the hypothesis and the value and benefits of such research.

The second chapter describes some of the factors, including human activities, which contribute to the pollution of the river with heavy metals and bacteria. A survey of anthropogenic activities in the Kahwa River catchment was used to identify sources of waste and its management.

The third chapter is an assessment of cadmium and lead pollution in samples of sediment and water from the Kahwa River. It describes the source of these metals in the river, the fate of metals discharged into the river and the health risk associated with these metals.

The fourth chapter assesses the impact and presence of bacteria in the Kahwa River sediment and water by describing relative levels of indicator and pathogenic bacteria in samples taken from the river. This chapter also describes the adaptation of the microbes to HMs and to antibiotics.

The last chapter is a general conclusion that is followed by the list of references.

CHAPTER 2: IMPACT OF HUMAN ACTIVITIES ON POLLUTION OF THE KAHWA RIVER

2.1. Introduction

Waste disposal constitutes a major urban problem worldwide (Javaheri *et al*., 2006; Ning, 2011). Unless properly managed, it may lead to irreversible pollution of ecosystems (Ntabugi, 2013). Such waste is generated in cities from household activities as well as from commercial and industrial sectors (Mukisa, 2009, Ning, 2011). It contributes, according to its composition, to the pollution of soil, air and water with microbes and toxic chemicals (Awomeso *et al*., 2010; Belaid, 2010).

Pollution of water has increased in cities in developing countries due to their demographic growth, economic development and the low educational level regarding waste management and environmental protection. In addition, urbanization structure may not allow easy collection of waste (Charnay, 2005). Thus, political authorities in developing countries face a major problem of waste management planning where the main difficulties they find are insufficient skilled persons in environmental management and in public administration, as well as corruption (Ntabugi, 2013). This motivates against effective waste management strategies (Aloueimine, 2006; Charnay, 2005).

Mutuku *et al*. (2014) reported that waste constitutes an important source of microbes and heavy metals (HMs) in urban river water. Contact with these pollutants leads to health risks (Nageswaran *et al*, 2012) including modification of the structures of enzymes and the evolution in microbes of resistance to antibiotics (Abdelatey *al*., 2011). Subsequent infection with antibiotic-resistant bacteria can compromise medical treatment and may increase patient mortality and morbidity (Yah and Eghafona, 2008). The consequences of the pollution of surface urban water could be reduced if the governments of developing countries prioritised the management of waste in governmental activities.

Waste is not managed in the Kahwa River catchment but is abandoned in the environment where it may contribute to environmental pollution by HMs and pathogenic bacteria. The current study aims to assess the impact of human

activities performed in the Kahwa River catchment on the pollution of sediment and water.

2.2. Review of literature

2.2.1. Waste management

2.2.1.1. Definition of waste

Waste is the abandoned residue of production processes and, by its nature, may contribute toxic effects into the natural environment (Citeretse, 2008; Ntabugi, 2013). According to Sane (2002), waste is all objects or substances with no economic value or is negative for the owner and who has to pay to have it taken away. The waste in the Kahwa River catchment that is produced from anthropogenic activities falls within the definition above.

2.2.1.2. The quantity of waste produced in urban cities

The quantity of waste produced in cities increases with improvement in the lifestyle of its citizens, in economic development and with population growth (Aina, 2006; Mukisa, 2009). Knowledge of the quantity of waste generated is important. It allows the planning of management systems to be applied to the waste produced so as to minimize environmental pollution (Francou, 2003; Charnay, 2005). Recent studies focussed on the quantity of waste produced in towns of both developing and developed countries (Charnay, 2005). The results obtained are presented in Table 1 and showed the difference in the quantity of waste produced in developing countries compared to, often, a relatively massive amount produced in developed countries.

Table 1 Relative increase in waste produced in developed countries (Adapted from Charnay, 2005 and Ntabugi, 2013)

Country	Town	Quantity of waste (Kg/habitat/day)
Brazil	Uberlandia	O.51
Cameroon	Yaounde	0.85
Malaysia	Kuala lumpur	1.70
China	Hong-Kong	0.7
Morocco	Rabat	0.60
USA	National average	1.8
Mauritania	Nouakchott	0.21
Mexico	Guadalajara	0.51
France	Paris	1.37
India	National average	0.41

2.2.1.3. Composition of urban waste

Urban waste is a mixture of materials characterised by different physical, chemical and biological properties (Francou, 2003).

Physical composition of waste: This knowledge allows defining the type of system to be applied to waste management (Aloueiminia, 2006).

Chemical composition of waste: Studies have been performed on the chemical composition of waste and aimed at determining the potential pollutants that may be found in the waste and the toxic effects they may have on human beings and their environment (Francou, 2003; Aina, 2005; Aloueminia, 2006; Ntabugi, 2013).

The physical and chemical properties of waste generated in various countries are presented in Table 2.

Table 2: Relative types and amounts of waste produced in various countries (adapted from Charnay, 2005)

Country	Organic waste (%)	Glass (%)	Plastics (%)	Paper (%)	Metal (%)
Benin	45	_	3-4	-	2
Burkina Fasso	39	3	10	9	4
Egypt	60	2.5	1.5	1.3	3
Guinea	69	0.3	22.8	4.1	1.4
Ile Maurice	68	1	13	12	1
India	38.6	1	6.03	5.57	0.23
Malaysia	36.5	3.2	18.4	27	3.9
Morocco	65-70	0.5-1	2-3	18-20	5.6
Mexico	55	4	4	15	6
Mauritania	48	3.8	20	3.6	4.2
Peru	34.7	7.1	7.2	6	2.8
Tunisia	68	2	7	11	4

Table 2 shows that organic, fermentable waste constitutes the major portion of waste that is generated in developing countries. In the DRC, such waste materials may lead to pollution of the Kahwa River with HMs and pathogenic bacteria.

Microbial composition of the waste: Microbes constitute the major health risk associated with waste generated from human activities (Mashhood and Mujahid, 2011). Humans may ingest these microbes after eating contaminated food or drinking contaminated water (Katarina and Payment, 2005). The presence of microbes in contaminated rivers like the Kahwa River can lead to microbial resistance to drugs and the proliferation of antibiotic resistance genes (ARG) in microbial biofilms (Spain and Alm, 2003; Mutuku *et al*., 2014). Diseases and symptoms associated with microbes that pollute water are indicated in Table 3.

Table 3: Diseases, agents and symptoms of waterborne diseases (adapted from Katarina and Payment, 2005).

Diseases	Agents	Symptoms
Bacterial diarrhoea	*Campylobacter jejuni*	Fever, diarrhoea, bloody stools
	E.coli	Fever, diarrhoea, bloody stools, uremic syndrome
	Salmonella spp	Mild gastroenteritis, acute diarrhoea, fetal septicemia
Typhoid fever	*Salmonella typhi*	fever, headache, appetite loss, nausea, diarrhoea, vomiting, abdominal rash.
Cholera	*Vibrio cholerae*	Watery diarrhoea, vomiting, occasional muscle cramps.
Legionnaire's Diseases	*Legionella pneumophila*	malaises, headache, fever, muscular aches, pains, cough, pulmonary symptoms.
Viral hepatitis	*Hepatitis A and E viruses*	Fever, jaundice, hepatitis, abdominal discomfort, chills, anorexia
Viral gastroenteritis	*Norovirus, Rotovirus,etc.*	Diarrhoea, discomfort, vomiting, malaise, fever, muscle aches, cough, chills.
Cryptosporidiosis	*Cryptosporidium parvum*	Diarrhoea, abdominal discomfort.
Giardiasis	*Giardia lamblia*	Diarrhoea, abdominal discomfort
Toxoplasmosis	*Toxoplasma gondii*	Swollen lymph glands, muscle aches and pain, congenital defects if mother infected
Amoebiasis	*Entamoeba histolitica*	Diarrhoea, abdominal discomfort

2.2.1.4. Disposal of waste in developing countries

Population growth, economic development and the urbanization structure in the towns of developing countries render the management of waste difficult (Francou, 2003 and Charnay, 2005). The majority of citizens discharge waste in and around the roads and rivers as it is important for them to place the waste away from their homes and work areas (Aina, 2005). Recent research carried out in developing countries revealed that 85 researched landfills did not respect environmental protection rules (Ntabugi, 2013). Johannessen *et al*., (1999) reported 97 discharges in Africa, Asia and South America and, of these, only 11 respected environmental rules. This is largely due to the fact that the siting of landfills in Africa is based upon ease and accessibility and, thus, escapes scientific

scrutiny. For example, waste discharge (landfill) in Akouédo, in Ivory Coast, is located in the region around the town around 47 km from the town itself. The choice of this site has been dictated by economic consideration rather than by scientific reasons and is located in a region where it may lead to water pollution (Sane, 2002; Ntabugi, 2013).

Research carried out on the Kahwa River catchment shows that the population of Bukavu Town discharges the waste from their activities into the environment and do not have sufficient information on the management of domestic waste (Ntabugi 2013). Mashhood and Mujahid (2011) reported that such waste disposal can lead to microbial pollution of drinking water. Such microbes may also affect humans by impacting on plant growth by competing with essential microbes of the rhizosphere (Wu *et al*., 2009).

2.2.1.5. Treatment of waste in developing countries

To minimize the impact of toxic compounds associated with waste such as HMs as well as infectious microbes, waste should be treated strictly in respect of environmental rules so as to protect the environment and human beings (Francou, 2003; Charney, 2005; Aina, 2006). Unfortunately, treatment of waste is mainly applied in developed countries and less so in developing countries. This is largely due to the fact that international conventions and protocols related to waste management are still absent in developing countries (Charnay, 2005; Aina, 2006). Such legislation involving dangerous waste management is included in the Bale convention signed on 5th December 2011, the transport of such waste in Africa is specified in the Bamako convention signed in 1999 and the persistence of organic pollutants is described in the Stockolm convention signed on 23th May 2001 (Charnay, 2005; Aina, 2006; Ntabugi, 2013).

The treatment of waste in developing countries varies from one country to another (Aina, 2006). The number of informal, non governmental organizations which are aimed at protecting the environment have increased in African countries. Unfortunately, these organizations have met with problems in their work including the poverty of citizens who cannot regularly pay for the service rendered

by the organization, urbanization which does not facilitate the collection of the waste and unexpected population growth (Ntabugi, 2013).

Solid waste collected in developing countries is often incinerated in an open dump, then composted and finally buried (Aina, 2006). Incineration of waste is most commonly applied in the treatment of waste as it allows a reduction in the volume of waste; it does not require large amounts of land space and kills the microbes associated with the waste (Francou, 2003, Charnay, 2005 and Aina, 2006). However, it can contribute to dissemination of HMs in the environment that can threaten the health of humans (WHO, 2007).

The study performed in the catchment of Kahwa River showed that solid waste is incinerated so as to reduce the waste volume. This may be effective in destroying microbes and in reducing the volume of waste. This waste treatment can increase the contamination of the sediment and water of the Kahwa River with cadmium (Cd) and Lead (Pb). Composting and burying of waste in the Kahwa River catchment is less frequently applied because the techniques require a large land space. Although composts may provide the advantage of supplying organic chemicals required for plant growth, they can be associated with microbes and HMs that can lead to water pollution. This may facilitate the accumulation of HMs in plants that can then be transmitted to human and animals through the food chain (Žukowska and Biziuk, 2008; Rajaganapathy *et al*., 2011).

2.2.1.6. Categories of water pollution

Water pollution occurs when a body of water is adversely affected due to the addition of large amounts of material (UN-Water, 2011). The way a water body is polluted may be categorized as being point source of pollution (PSP) or non-point source of pollution (NPSP) (Mubwebwe, 2009; Bagalwa *et al*., 2013). The PSP may occur when pollutants are emitted directly into waterways from specific locations such as drain pipes, ditches, sewer outfalls, factories, power plants, sewage treatment plants, underground coal mines and oil wells. Such pollution is discrete and relatively easy to monitor and to treat particularly when discharges are relatively uniform throughout the year (Mubwebwe, 2009). On the other hand, a NPSP may be scattered and have no specific location of discharge into water

bodies. Such NPSPs include farm fields, lawns and gardens, construction sites, logging areas, roads, street, parking, atmospheric deposition, landfill and agricultural pathogens. These sources are often highly episodic and the concentrations of contaminants are not uniform and so are difficult to monitor, regulate and to treat (Mubwebwe, 2009).

The pollution sources of the Kahwa River and its tributaries may be classified as consisting of both NPSPs and PSPs. It includes contaminated waste from healthcare facilities, construction debris, refuse from street cleaning, park and landscaping activities, sewage and landfill effluent and wastewater. The effect of water pollution can contribute to poisoning of water and food and to disruption of the balance of the Kahwa River and Lake Kivu. The effects and sources of some pollutants in water are indicated in Table 4.

Table 4: Effects and Sources of Pollutants in water (adapted from Mubwebwe, 2009)

Pollutant	Source	Effects
Nutrients	Municipal and rural wastewaters Run-off from agriculture Industrial discharges Aquaculture operations Forestry Atmospheric emissions and deposition	Eutrophication Health effects in drinking water
Salts/ Salinization	Poor drainage High evaporation rates Over-pumping of coastal aquifers Irrigation Clearing of natural forests for agriculture	Aesthetic value of water lowered Agricultural land lost Difficult to remedy Damage to pipes and pumps
Organic Wasters	Domestic sewage Industrial sewage	Oxygen depletion Complexing into carcinogens
Organic micro-pollutants	Industry Urban and agricultural wastewater runoff Atmospheric fallout Solvents and aerosols	Immediate short term toxicity Long term exposure to toxic compounds and carcinogens
Faecal material	Domestic sewage Storm water drainage Onsite sanitation contamination	Contain pathogens leading to water-borne infections
Toxic compounds/ Heavy metals	Processing of ores Industrial use of metals Leaching from dumps	Bio-accumulation in aquatic organisms Heavy metal poisoning
Atmospheric Emissions	Fossil fuel combustion	Acid rain decreases water and soil pH Impair reproduction of aquatic life Human exposure to toxic metals leached from soils
Sediments/ Turbidity	Soil erosion Organic formation within a water body Human activity by-products	Impairment of aquatic life Increased costs of treatment

2.2.1.7. Risk associated with waste

A large proportion of waste in developing countries consists of organic, fermentable waste (Table 2) which can promote the growth of microbes and the breeding of vectors of diseases such as malaria, typhoid fever, shigellosis and cholera (Charnay, 2005; Carr and Neary, 2008; Obasohan *et al*., 2010). In

addition, fermentable waste discharged into aquatic ecosystem may lead to eutrophication (Carr and Neary, 2008). Eutrophication is linked to excessive accumulation of nutrients in the water that then decompose by the action of aerobic microbes resulting in biological, chemical and physical changes in the water body. Biological effects manifest with excessive growth of algae and cyanobacteria (Muvunja, 2010). Algae are observed at the surface of water where the energy from the sun's rays is captured followed by the production of oxygen. However, the decomposition of waste and dead algae by aerobic bacteria is followed by a decrease in oxygen within the water. This increases the relative anaerobic status of the water and reduces the aerobic life support capacity of the water (Baddi *et al*., 2004). This favors the multiplication of bacteria beneath the surface of the water and that of algae at the water surface. This algal distribution can reduce recreational activities, the movement of boats and lead to a reduction in tourism revenues (Garland et Mills, 1991; Carr and Neary, 2008; UNICEF, 2008). In addition, eutrophication changes the physical characteristics of water such as a change in the water color that may inhibit the penetration of the sun's rays into the water (Ntabugi, 2013).

The chemical effects of eutrophication involve the reduction of dissolved oxygen in the water due to excessive aerobic bacterial activity (Muvunja, 2010). When HMs are introduced into aquatic environments, they may bind to organic particles and the decomposition products of waste material by bacteria in the water. This may allow their uptake by the microbes, followed by the utilisation of oxygen (Ntabugi, 2013). This process can also increase the concentration of protons (H^+) in water leading to a reduction of the pH of water that can affect aquatic life (Carr and Neary, 2008; UNICEF, 2008).

2.3. Methodology

2.3.1. Sampling

From June to August 2013, research involved the completion of a questionnaire (Appendix A). This involved families and work areas in the districts of Bukavu Town (Ibanda, Kadutu and Bagira) situated in the Kahwa River catchment in order to investigate the possible pollution sources of the Kahwa River regarding bacteria and HMs, particularly Cd and Pb. Individuals and families in these districts were selected according to the following inclusion criteria: (1) they generate waste and wastewater and (2) the waste and wastewater may be deposited into the Kahwa River.

The size of the population in the selected avenues and streets is estimated at 406,118 persons (The Mayor of Bukavu Town, Fourth trimester, 2013). The number of families in the streets involved in this research was determined as guided by Ntabugi (2013). Research carried out on sub-Saharan African populations revealed that a family consists of an average of 7 persons. In order to find the number of the families in the streets concerned by the research, the size of this population (406,118) was divided by 7. Thus, the number of families in the current research was then estimated to 58,016 families. This latter figure was considered as being the research population. According to Depelteau (2001), when the size of the research population is between 50,000 and 75,000, a sample size of 382 should be chosen. This figure was considered as representing 0.6584293% of the 58,016 families, as illustrated in the following formula:

Percentage of the sample (P) = $\frac{\mathbf{TS \times 100}}{\mathbf{TN}} = \frac{\mathbf{382 \times 100}}{\mathbf{58016}} = \mathbf{0.6584293\%}$

Where**,**

TS: sample size;

TN: total number of families.

In order to find the sample size per street, the percentage of the sample “P” was adapted in the street as showed in the following formula:

The sample per street = $\frac{\mathbf{TN \times P}}{\mathbf{100}} = \frac{\mathbf{TN \times 0.6584293}}{\mathbf{100}}$

Where,

TN: Number of families per street

P: percentage of the sample

The sizes of samples per street in the district of Bukavu Town are presented in Table 5:

Table 5: Size of sample per avenues in the districts of Bukavu Town

Districts	avenues	Streets	Population/ street	Family number/ street	Sample/street
Kadutu	Cimpunda	Nyamulagira	13760	1965	(13) 12,94283927
		Elila	15753	2250	(15) 14,81748162
		Sake	11139	1591	(10) 10,47749176
	Kajangu	Burhalaga	5470	781	(5) 5,145154856
		Busoka	11185	1597	(11) 10,52075998
	Kasali	Ulindi	7758	1108	(7) 7,297278131
	Mosala	Buholo	44595	6370	(42) 41,94665097
		Funu	16550	2364	(16) 15,56715043
		Karhunva	14627	2089	(14) 13,75835102
	Nkafu	Kahwa	19480	2782	(18) 18,32314746
		Clinique	10767	1538	(10) 10,12758361
	Nyakaliba	Kahuzi	15732	2247	(15) 14,79772874
		Mulima	25090	3584	(24) 23,59998818
	Nyamugo	Lomami	43132	6161	(41) 40,57053369
		Utu	18395	2627	(17) 17,30258201
		Byasi	9671	1381	(9) 9,096671411
Ibanda	Ndendere	Kibombo	12980	1854	(12) 12,20916088
		Route d'Uvira	17728	2532	(17) 16,67519292
	Panzi	Major Vangu	17322	2474	(16) 16,29330392
Bagira	Kasha	Buholo	35442	5063	(33) 33,33721726
		Chahi	13593	1941	(13) 12,78575685
		Ciriri	19592	2798	(18) 18,42849615
		Mulwa	6357	908	(6) 5,979478871
TOTAL			**406118**	**58016**	**382**

After applying the formula to find the number of samples per street, the result was rounded off to the nearest integer as presented in table 5.

The healthcare facilities in the Kahwa River catchment were also selected as sites of wastewater and run off flow into the Kahwa River. The healthcare facilities selected per district are presented in Table 6:

Table 6: Healthcare centres per district in Bukavu Town

Districts	Health cares	Health care location
	CBCA heath centre	Nyamugo
	CECA health centre	Camps Mweze
Kadutu	8th CEPAC centre	Buhololl
	Catholic heathcentre/Funu	Funu
	Maendeleo health centre	Cimpunda
	Bishop Emmanuel Kataliko health centre	Biname
	Bishop Mulindwa health centre	Nyamulagira
	Medical centre Saint Vincent	Kasali
	Neema health centre	Kasali
	Uzima heath centre	Rukumbuka
Ibanda	CELPA hospital centre	Avenue de la 10th region militaire
	Maman Mwilu health centre	Major vangu
	Neema health centre	Route d'ouvira
Bagira	Cahi hospital centre	Chahi
	Lwazo health centre	Camps regie
	Ciriri health centre	Ciriri
	Ciriri reference hospital	Ciriri

Table 6 shows that most of the healthcare facilities were located in Kadutu and it is expected that this district contributes significantly to the pollution of the Kahwa River.

The garages in the area are an organization of 3 to 5 citizens working together along the road. The criteria used in selecting garages for inclusion in this study were the following: (1) the garage should have five workers; (2) its wastewater and runoff may drain into the Kahwa River. A list of selected garages is presented in Table 7.

Table 7: Garages of the three Districts of Bukavu Town

Districts	Garage names	locations
Kadutu	Deuxpoteau	Deux Poteau/Nya
	Cheche	Bugabo I
	CAPA	Industriel
	ONL	ONL/Cimpunda
	ITFM	ITFM
	Buholo VI	Buholo 6
Bagira	Zaire Cep	Essence
Ibanda	Aumonerie/ISP	Aumonerie/Kibombo

Table 7 shows that most of the garages that pollute the Kahwa River are located in Kadutu.

2.3.2. Data analysis

The data presented in the completed questionnaires were analysed using the software package Epi-info to provide answers by the citizens to determine the frequencies of activities and details of waste removal.

2.4. Results and discussion

The current study focused on the pollution sources affecting the Kahwa River. The results obtained from the investigations in the garages, healthcare facilities, soap factories and families, followed by the discussion, are presented in the following sections.

2.4.1. Families

2.4.1.1. Types of Domestic waste

The results of the questionnaire indicated that waste generated in families consists of batteries (79.8%), cooking oil (25.4%), the remains of food (69.1%), plastics (75.9%) and glass (24.1%). This is indicated in Figure 1. Rai *et al*. (2010) and Ntabugi (2013) reported that domestic waste, by its composition, may contain HMs and bacteria.

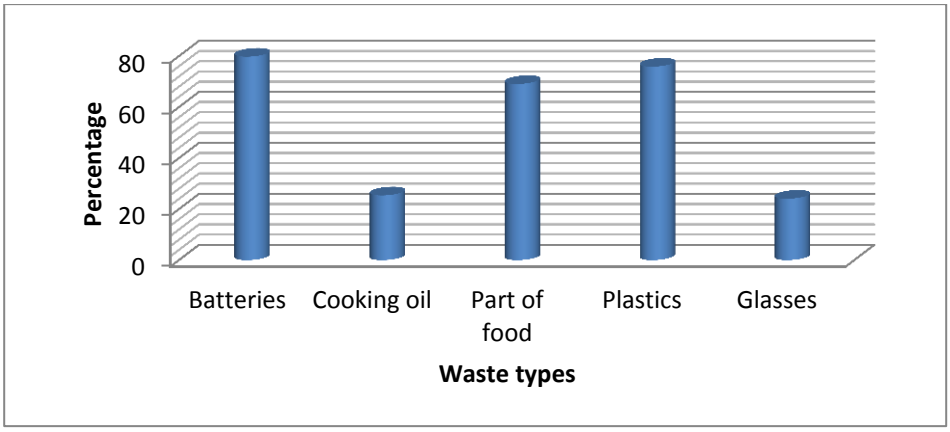

Figure 1: Types and relative amounts of waste generated by families (presented as a %).

2.4.1.2. Disposal of solid domestic waste

Figure 2 shows that solid waste produced in the families is discharged into canals (34.5%), on the ground (21.3%), into landfills (27.4%) and into rivers (16.8%). The domestic solid waste may contain bacteria, nutrients and HMs such as Cd and Pb (Belaid, 2010, Rai *et al*., 2010). Its discharge into the canals and the rivers in the Kahwa River catchment can contribute to microbial and HM pollution of the Kahwa River and Lake Kivu. In addition, solid waste accumulated in the river may provide the ideal breeding environment for bacteria and disease vectors contributing towards diseases such as malaria, typhoid fever, cholera and dysentery (Katarina and Payment, 2005 and Obasohan *et al*., 2010). Furthermore, nutrient loading into the river contributes to euthrophication which can deplete the oxygen in the water (Muvunja, 2010). Thus, the waste discharged in the Kahwa may favor the multiplication of bacteria and may exacerbate Cd and Pb pollution in the sediment and water in the river.

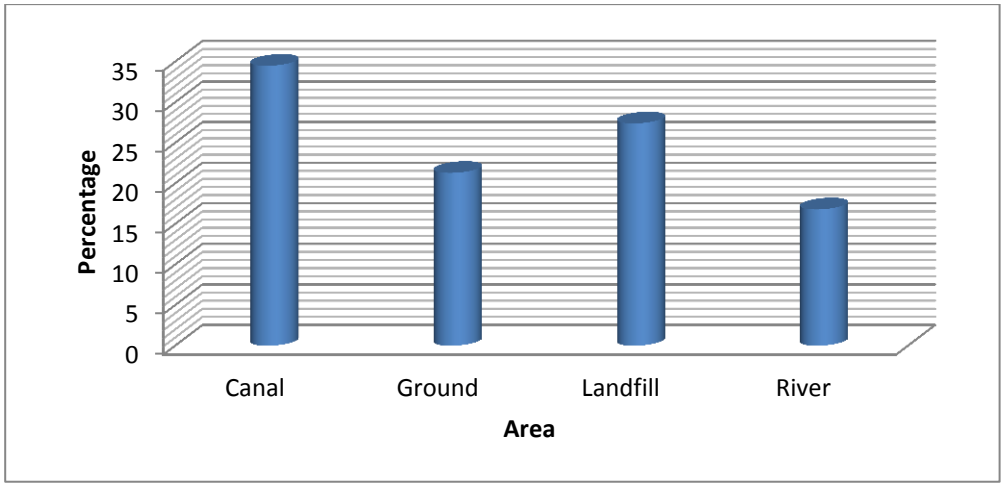

Figure 2: Routes of disposal of domestic solid waste (presented as a %).

2.4.1.3. Treatment of landfill waste

Figure 3 shows that solid waste transported to landfills is either incinerated (86.5%) or composted (13.5%). Incinerated waste can release Cd and Pb into the environment (WHO, 2007) that can return in the nearby water body through runoff and rain water (Nacklé, 2003 and Blinda, 2005). Thus, the incineration of waste in the catchment of Kahwa River may pollute river sediment and water with HMs.

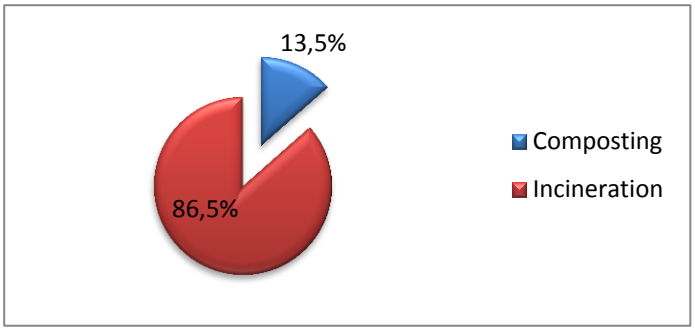

Figure 3: Treatment of waste disposed of in landfills (presented as a %).

2.4.1.4. Wastewater disposal

Domestic wastewater is discharged into the canals (69.4%), on the ground (21.8%) and into the rivers (8.9%) This is indicated in Figure 4 and confirms results obtained by Ntabugi (2013). Wastewater may contain microbes, antibacterial agents and HMs (Cd and Pb) (Belaid, 2010).Thus, the wastewater discharged in the Kahwa River catchment can contribute to its pollution.

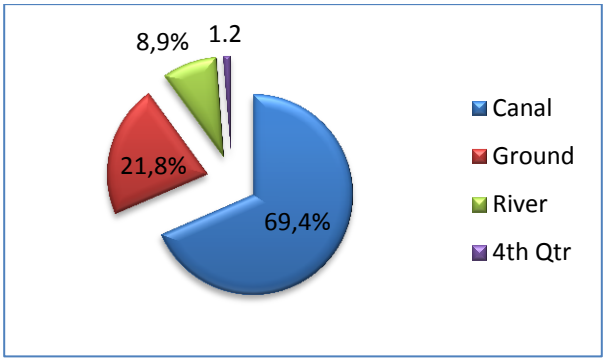

Figure 4: Routes of discharge of domestic wastewater indicated as a %

2.4.1.5. Toilet waste

Figure 5 shows that families make use of toilets that are connected to a canal (21.2%), a river (6.3%) or a septic tank (4.5%) but are mainly associated with a pit latrine (68%). The toilets connected to canals and to rivers may contribute to microbial pollution of the Kahwa River (Katarina and Payment, 2005; Lotter, 2010).

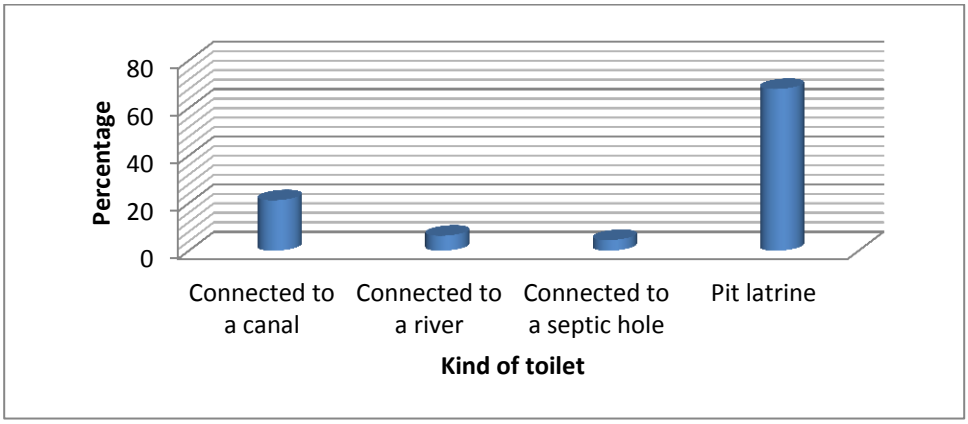

Figure 5: Types of domestic toilets (presented as a %).

2.4.2. Healthcare facilities

2.4.2.1. Healthcare services

The study revealed that healthcare facilities provide the following services: pediatric care (62.5%), surgical care (68.8%), radiography (68.8%) and nephrology (6.3%), as shown in Figure 6. In addition, 94.1% of these facilities have inpatients and generate waste that includes batteries (67.5%), antibiotics (100%), blood (100%), dyes and stains (100%), faecal matter (100%), urine (100%) and syringes (100%). According to ICRC (2011), Kamalakanta and Akilesh (2012), Prüss *et al*.,

(2013) and Rahele and Givindan (2013), the services organized in the healthcare facilities generate waste that can contain both bacteria and HMs such as Cd and Pb.

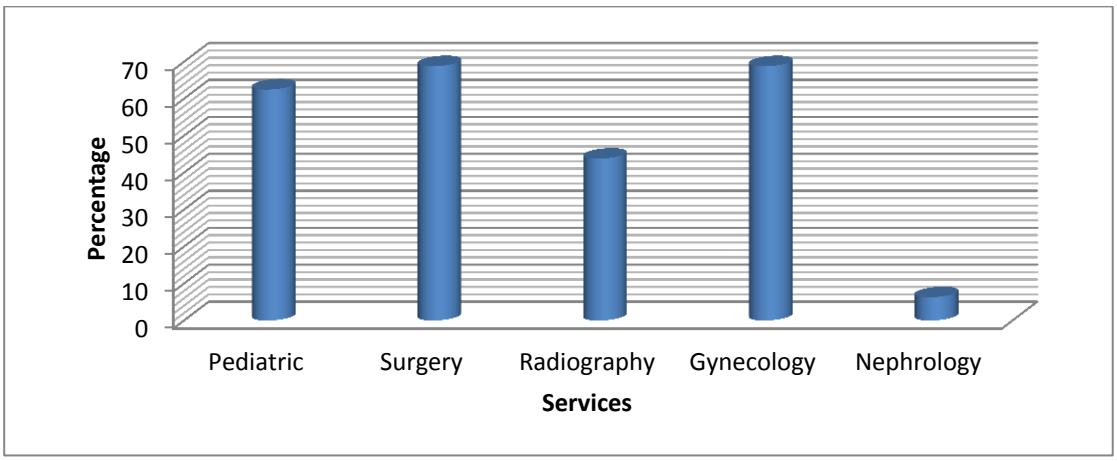

Figure 6: Services performed in the healthcare facilities

2.4.2.2. Disposal of healthcare waste

Figure 7 shows that waste generated from the healthcare facilities are discharged either into canals (43.7%) or septic holes (56.3%) before being disinfected. The healthcare wastewater consists of antibiotics, microbes, urine, faecal matter, stain and dye which are known to contain bacteria and HMs (Prüss *et al*., 2013). This wastewater that is discharged into the environment may lead to pollution of the Kahwa River with antibiotic-resistant microbes, antibiotics and HMs. Czekalski *et al*. (2012) and Mudryk (2002) reported that healthcare waste is the major contributor of antibiotics in the environment. In addition, bacteria evolving in areas containing antibiotics may become antibiotic resistant (Tortora *et al*., 2010; Kohanski *et al*., 2010).Solid waste from the healthcare facilities is incinerated in open dumps. Incineration of waste can lead to pollution of the environment with Cd and Pb through runoff and rain water (Blinda, 2005); Kamalakanta and Akilesh 2012).

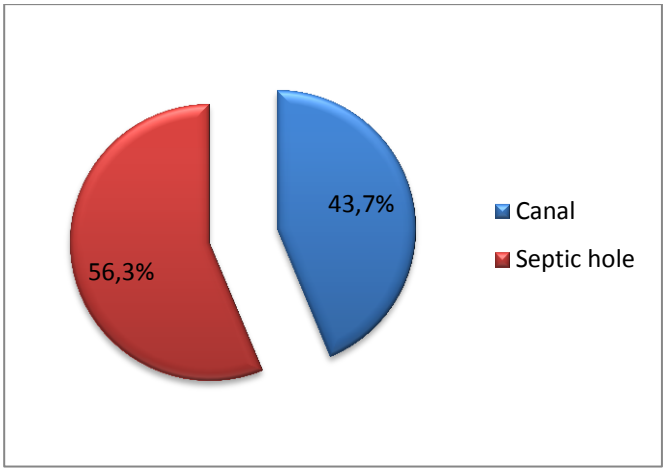

Figure 7: Methods of disposal of healthcare waste (presented as a %)

2.4.3. Garages

2.4.3.1. Activities in the garages

Welding (100%), painting (100%), motor revision (100%) and car washing (75%) constitute the main activities performed in the garages (fig. 8). According to the interviewees, the waste generated in the garage activities is not treated before being discharged. It can then lead to Cd and Pb pollution of the sediment and water in the Kahwa River.

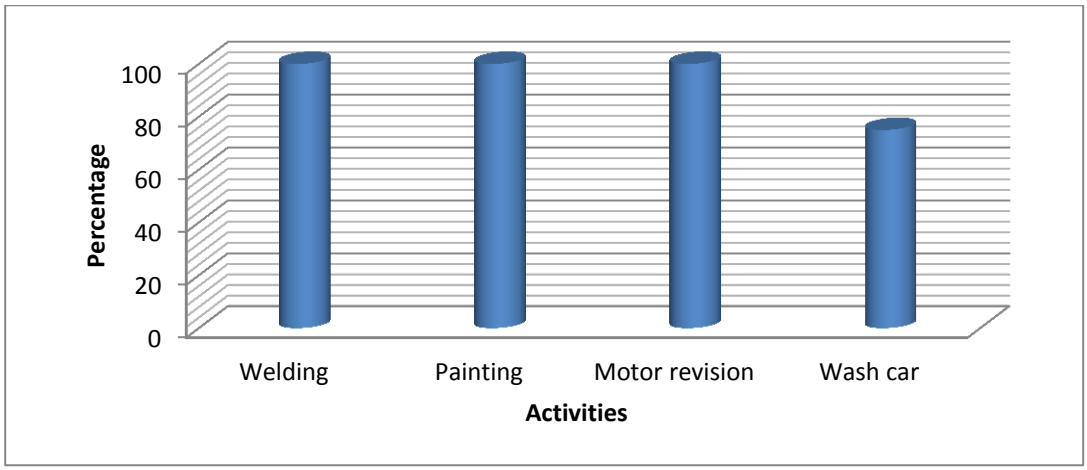

Figure 8: Main activities in garages in Bukavu Town

2.4.3.2. Waste generated in garages

The materials used in the garages to subsequently generate waste include dye (100%), used tires (28.6%), paper (75%), wastewater (75%), metals (75%) and used oil motor (100%). This is shown in Figure 9. According to Sekomo

(2010), the garage wastewater contains Cd and Pb and so its discharge into the Kahwa River catchment or directly into the river can lead to pollution of the river.

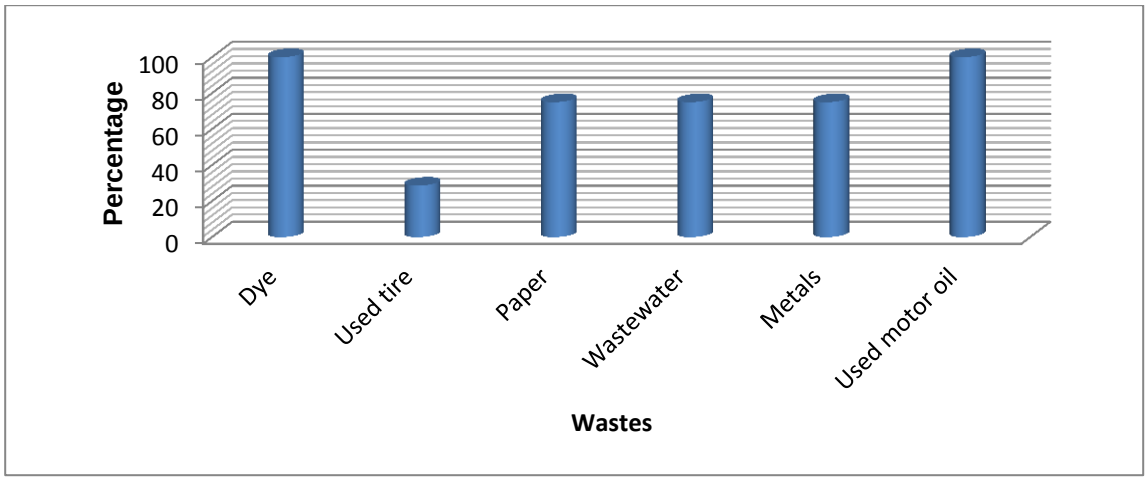

Figure 9: Types of waste produced from garage activities

2.4.3.3. Garage waste disposal

Figure 10 shows that the waste generated from garage activities is discharged on the ground (50%) or into canals (25%) or the river (25%). Most of the garages in the area were located near the rivers, streams or the canals to allow easy waste disposal. This observation is similar to that reported by Adelekan and Abegunde (2011) in Nigeria.

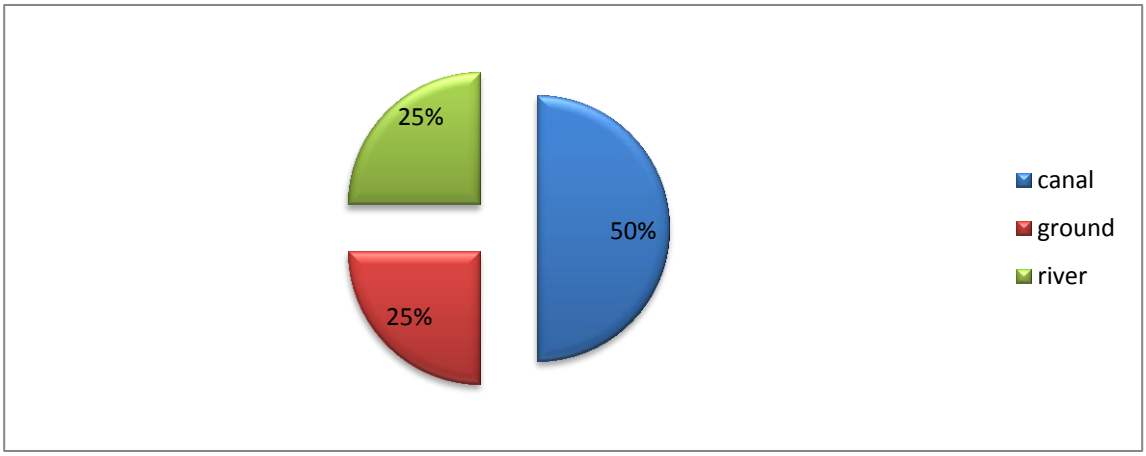

Figure 10: Relative disposal of garage waste (presented as a %).

2.4.4. Conclusion

The current research was carried out in Bukavu Town from June to August 2013. It aimed to identify possible source of pollution of the Kahwa River with bacteria and HMs, particularly Cd and Pb. Investigation in the form of a questionnaire (Appendix A) was performed to determine the type and disposal of waste from domestic households, healthcare facilities, garages and soap factories situated within the river catchment so as to assist in determining contamination of the Kahwa River and, subsequently, Lake Kivu. In order to achieve the research goals, the questionnaire was distributed to interviewee sites and families that were sampled and selected according to Depelteau (2001). The relatively few questionnaires submitted to healthcare facilities, factories and garages were all returned for analysis.

It was found that domestic wastewater is mainly discharged into canals (69.4%), on the ground (21.8%) and the remainder is discharged into the river (8.9%). The vast majority of domestic solid waste is incinerated (86.5%). Domestic toilets are connected to a canal (21.1%), rivers (6.3%) or septic hole (4.5%) while the majority of toilets are linked to pit latrines (68%). The solid waste generated from healthcare facilities is incinerated (100%), while the wastewater is released either into septic holes for sterilisation (56.2%) or into canals (43.8%). Factory waste is released into canals while wastewater from garages is evenly disposed of: into canals (37.5%), on the ground (37.5%) and the remainder into rivers (25%).

These results indicate that waste generated from anthropogenic activities sited along the Kahwa River catchment can contribute to an increase in contamination of river sediment and water with HMs (Cd andPb) and bacteria. Such dual contamination may have adverse effects on the Bukavu population and the biodiversity through infection by ARB and the bioaccumulation of these toxic metals, known to disturb metabolic processes. It is, therefore, important that citizens and urban authorities involved in the management of waste generated in the area should protect the river from pollution.

CHAPTER 3: ASSESSMENT OF CADMIUM AND LEAD IN THE KAHWA RIVER

3.1. Introduction

Population growth in the Kahwa River catchment has led to an increase in waste discharged into the environment (Bagalwa *et al*., 2013). Most of these waste materials are neither collected nor treated but is delivered directly into the environment where it is likely to contribute to pollution of air, soil and water. Together with traffic vehicles and the associated combustion of fossil fuel, sites contributing to the production of waste include markets, domestic households, factories, cosmetic and paint workrooms, garages and healthcare facilities. The waste resulting from these human activities is either incinerated or dumped into canals, rivers, along the roads or onto the ground. Pollution of river water and sediment with Cd and Pb may result from rain water runoff flowing onto soil, from erosion and the leaching of rocks, as well as wastewater and dumped waste (European Commision DG ENV E3, 2002; WHO, 2007; Sakultantimetha *et al*., 2009). As a result, humans and animals exposed to such river water may develop cancer, growth defects and metabolic disorders (Vasilelos, 1998; Zakir and Shikazono, 2011).

Heavy metals constitute the major environmental problem because they can initially accumulate in organisms and transfer to other beings through the food chain (Žukowska and Biziuk, 2008; Rajaganapathy *et al*., 2011). Secondly, they are difficult to eliminate in the environment as they do not degrade as do many organic pollutants (Harman *et al*., 2007; Aderinola *et al*., 2009; Reza and Singh, 2010; Wogu and Okaka, 2011). Thirdly, they cause various diseases and metabolic disorders (Nasrazadani *et al*., 2011). Some HMs like iron (Fe), calcium (Ca) and Magnesium (Mg) are essential micronutrients (Reza and Singh, 2010; Oronsaye *et al*., 2010; Wogu and Okaka, 2011). They become toxic, however, if accumulated in high concentration (Cubaka, 2010; Akan *et al*., 2010). Others, such as Pb and Cd, are toxic to all the organisms and do not have any known physiological role (Mwashote, 2003; WHO, 2007; Akan *et al*., 2011). Exposure to these metals may occur through inhalation of metal particles, via ingestion through contaminated food, drinking water and via body contact with the contaminated environment (Blinda, 2005; WHO, 2007; Prasad, 2009). Once these HMs enter an

organism, they affect biomolecular structure and disturb metabolism (Abdelatey *et al*., 2011). They may cause sub-lethal effects within organisms including histological or morphological change in tissues, suppression of growth and development and changes in circulation, behavior and reproduction (Nacklé, 2003; WHO, 2007; Akan *et al*., 2010).

A critical assessment of Cd and Pb pollution of Kahwa River is important to ensure the protection and restoration of water quality in both Lake Kivu and the Kahwa River in order to protect the health of the population and aquatic life from the adverse effects of such pollution. This aim governs the current study.

3.2. Review of literature

3.2.1. The fate of Cd and Pb in the Kahwa River

Heavy metals such as Cd and Pb are discharged into the Kahwa River from anthropogenic activities performed within the river catchment. Mahmud *et al*. (2012) reported that heavy metals discharged into aquatic environment may bond with organic molecules and together these precipitate to the bottom of the river and add to the sediment. Secondly, they can dissolve in water which transports them into ground water leading to the pollution of aquifer water (Sekomo *et al*., 2009; Mahmud *et al*., 2012). Thirdly, in Bukavu Town, the metals may either remain in the river or be carried into Lake Kivu where they may be absorbed and accumulate in aquatic organisms (Rajaganapathy *et al*., 2011). Humans and the biodiversity can then take up these HMs through the food chain in the process of biomagnification (Nacklé, 2003; Žukowska and Biziuk, 2008). Bioaccumulation of these metals depends on the species involved and the physical and chemical characteristics of the metals (Blinda, 2005; Sekomo, 2010).

Bacteria evolving in a Cd- and Pb-polluted environment may develop mechanisms to withstand HMs pollution (Hynninen, 2010; Intorne *et al*., 2012). These mechanisms include the efflux of metal ions to the outside of the cell, accumulation and complexation of metal ions inside the cell, and the reduction of the heavy metal ions to a less toxic state (Anyanwu and Ugwu, 2010; Mutuku *et al*., 2014). In addition, recent research revealed a correlation between bacterial

resistance to HMs and to antibiotics (Spain and Alm, 2003 and Barifaijo *et al*., 2009). Resulting resistant bacteria may lead to antibiotic resistance within the microbial biofilm and this can compromise the health of humans and the biodiversity through infection leading to an increase in the mortality and morbidity rate (Abdo *et al*., 2010; Czekalski *et al*., 2012). Regarding the food chain, HMs may accumulate in the lake plankton and aquatic invertebrates before being eaten by fish that, in turn, are consumed by humans (Rajaganapathy *et al*., 2011). The accumulation of HMs into internal human organs can affect growth, the multiplication of cells and damage to the nervous system and internal organs (Mwashote, 2003; Okaka and Wogu, 2010).

Diseases related to HMs pollution of the Kahwa River may compromise the development of Bukavu Town. Knowledge of the bacterial and HMs loading of the Kahwa River and it catchment should convince decision makers to sensitize the population and relevant organizations to reduce the pollution of sediment and water in the Kahwa River.

3.2.2. Interaction between bacteria and heavy metals in surface water

3.2.2.1. Introduction

Worldwide, urban rivers receive effluent from families, industries and workrooms that contaminate the sediment and water with HMs and bacteria (Rai *et al*., 2010). Bacteria evolving in such polluted rivers interact with metals either to their benefit or to their detriment (Nies, 2003; Ehrlich, 1997). Some heavy metals like copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn) and sodium (Na) are important micronutrients (Cubaka, 2010). However, others like Cd and Pb have no known biological role in microbes (Nies, 1999). Their presence in bacteria is toxic because they can disturb metabolic functions by binding as ligands to nucleic acids and essential proteins or by displacing essential metals from their natural binding site in the cell (Ehrlich, 1997; Nies, 1999, Hynninien, 2010). For example, Cd and Pb can bind sulfhydryl groups and, thereby, inhibit enzymatic activities. Furthermore, they can bind glutathione leading to the formation of bis-glutathione (Nies, 1999; Bruins *et al*., 2000). In order to withstand

Cd and Pb contamination, bacteria have evolved resistance mechanisms as described in the following section.

3.2.2.2. Bacterial resistance to Cadmium and Lead in aquatic environments

Cadmium and lead enter bacterial cell by diffusion and their bioavailability may result in bacterial death (Ehrlich, 1997; Nies, 1999; Hynninen, 2010). Resistance to these metals is mainly based on active efflux of metal ions, and binding protein factors (BPF) that prevent toxicity of the metals (Ehrlich, 1997; Hynninen, 2010). The efflux of metals from the cytoplasm of bacteria is facilitated by proteins such as P-type ATPases, Cassette Binding ATP transporters (CBA) and the Cation Diffusion Facilitator **(**CDF) transporters present in the plasma membrane (Cubaka, 2010; Hynninen, 2010).

The P-type ATPases constitute the group of protein carriers located in the plasma membrane of bacteria. They transport Cd^{2+} and Pb^{2+} against the concentration gradient from the cytoplasm toward the periplasm (Nies, 1999; Hynninen, 2010). The P-type ATPases involved in the HMs homeostasis belong to PB1-ATPases. These proteins play two important and essential roles: they provide essential HMs required in metalloproteins maturation firstly and secondly they move toxic metals like Cd^{2+} and Pb^{2+} through bacterial membrane from the cytoplasm of bacteria to the environment (Hynninen, 2010). The HMs transporters ATPases (HMTA), also called CPx-type ATPases, conserve proline residue (P) preceded or followed by cysteine residues (C) in its structure (Cubaka, 2010; Hynninen, 2010). CPx-type ATPases transport, especially, Cd and Pb ions from the bacterial cytoplasm to the periplasm without further transport to the bacterial external environment (Nies, 1999; Hynninen, 2010). The specificity of HM-translocating ATPases is determined by metal binding domains (MBD). The PB1-type ATPases have two MBDs: the first is located on the N-terminal cytoplasmic domain and the second is situated on the transmembrane segment. The well known pumps that probably expel Cd^{2+} and Pb^{2+} from the cytoplasm of bacteria include CadA, ZntA, and CopA (Hynninen, 2010).

The Cassette Binding ATP (CBA) transporters consist of three proteins that span the whole cell wall of gram negative bacteria. The first is the resistance

modulation and cell division superfamily (RMD); the second is the membrane fusion protein (MFP) and the third is the outer membrane factor (OMF) (Hynninen, 2010).The RMD protein is the important component of the CBA efflux system mediating the active part in the transport processes of the metals. In addition, it determines substrate specificity and it is involved in the assembling of trans-envelope protein complex. Furthermore, the RMD protein, located in the bacterial inner membrane, is involved in resistance to HMs and cell division. It is usually accompanied by the MFP and the OMF (Cubaka, 2010). These proteins, taken together, form the efflux protein complex that exports HMs from the cytoplasm, the plasma membrane and the periplasm of a bacterium to its external environment (Nies, 2003). They protect the bacterium from the adverse effects that may be linked to Cd and Pb accumulation in its cytoplasm.

The cation diffusion facilitator (CDF) consists of a group of bacterial plasma membrane protein transporters involved in the regulation of HMs. These catalyze the efflux and the influx of heavy metals (Cubaka, 2010). The export of Cd^{2+} by the CDF has been reported in *C. metallidurans*, *S aureus* and *E. coli* (Hynninen, 2010). Proteins involved in the regulation of HMs concentration in bacterium are presented in Figure 11.

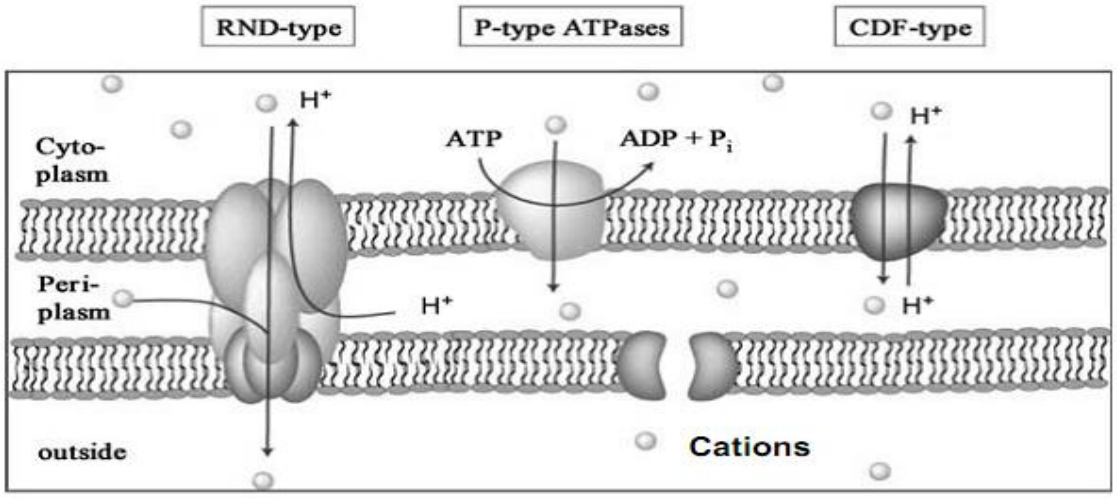

Figure 11: Proteins involved in the control of HMs in bacteria (adapted from Cubaka, 2010).

Binding protein factors (BPF) are a group of proteins that detoxify by sequestering HMs that are available in the bacterial cytoplasm. The BPF consists of the intracellular binding proteins (IBP) formed from bacterial metallothioneins (BMs), bacterial metallo-chaperones (BICs) and the cell wall components (CWC) (Hynninen, 2010). The BMs are small proteins that lower free ion concentration in the cytoplasm of a bacterium. They are composed of molecules of cysteine that bind Cd^{2+}. The first metallothionein characterized in bacteria, the SmtA, sequesters and detoxifies Cd^{2+}. In *E coli*, proteins that form Cd^{2+}/Pb^{2+} binding metallo-chaperones include periplasmic protein ZraP. It is only produced when the cells are incubated with high concentrations of divalent cations (Hynninen, 2010).

The main role of metallo-chaperones is the sequestration of toxic metals such as Pb and Cd in order to protect the periplasm and the transmembrane proteins and to allow cooperation between proteins involved in resistance to HMs (Hynninen, 2010). Several bacterial species use Cd and Pb intracellular and extracellular binding proteins to avoid toxicity. For example, *Salmonella aureus* and *Vibrio* lower the concentration of free Pb ions by precipitating it as extracellular polymer (Hynninen, 2010).

3.2.3. Characteristics of Cadmium

3.2.3.1. Introduction

Cadmium, a natural element of the earth's crust, is relatively soft and occurs in the natural environment associated mainly with zinc ores and, to a lesser extent, with lead and copper ores (Nacklé, 2003 and Blinda, 2005). In natural surface and ground waters, Cd is found in the form of Cd^{2+} ions. It can also exist as hydrated ions, inorganic complexes with carbonate, hydroxide, chlorides, and sulfate or as organic complex with humic acid (UNEP, 2008).

Cadmium is not essential to plants, animals, humans or to microorganisms but rather it is harmful to all organisms as it inhibits the activities of enzymes and may disturb the structure of DNA and normal metabolism (UNEP, 1998; Nacklé, 2003). Plants absorb Cd from its environment, in the form of Cd^{2+} ions, through the roots and leaves by diffusion (Miquel, 2001). Once absorbed, it is transported throughout the plant (Guns and Pussemier, 2000; WHO, 2007). Green plants are

the starting link of the food chain within ecosystems and are, therefore, the main source of Cd for animals and humans (Guns and Pussemier, 2000; Ekpo *et al*., 2008). Cadmium-contaminated foods, resulting from the uptake of contaminated water from the soil, can cause the bioaccumulation of Cd in terrestrial and aquatic animals (Ekpo *et al*., 2008).

The monitoring of Cd in the Kahwa River is important as it should help to reduce adverse risks resulting from ingestion of such contaminants from the environment.

3.2.3.2 Source and use of Cadmium

Cadmium is released in the environment via natural occurrences (volcanic eruption, weathering and erosion, the vegetation, airborne particles and forest burning (UNEP, 2008). Human activities like tobacco smoking, mining, smelting and refining of nonferrous metals, fossil fuel combustion, incineration of municipal waste containing Cd, manufacture of phosphate fertilizers, recycling of cadmium-plated steel scrap, electric and electronic waste, domestic wastewater, domestic solid waste and paint dye are also sources of Cd (Nacklé, 2003; Blinda, 2005 and WHO, 2007). Importantly, garage waste and wastewater is an important source of Cd (Sekomo, 2010).

In addition, Cd ions and Cd compounds have found increased applications in industrial products and operations increasing in the spread of this metal in the environment. These activities include:

Electroplating: cadmium is deposited either electronically or mechanically onto objects to provide a bright appearance and resistance to corrosion.

Pigment: cadmium pigments are used in the plastic industry, ceramics, paints and in the glass enamel, red-labeled ‘’Coca Cola’’ bottles;

Plastic stabilizers: cadmium stearates are used as stabilizers in the production of polyvinyl chloride plastics (PVC);

Batteries: due to its perfectly reversible electrochemical reactions at a wide range of temperatures, it’s low rate of self-discharge, and the easy recovery from dead batteries, Cd is employed extensively in battery manufacture;

Other use of Cd are: i) cadmium phosphors are found in the tubes in television sets, fluorescent lamps, X-ray screens, cathode-ray tubes, and phosphorescent tapes, ii) cadmium alloys in Cd-Ag solders, automatic sprinkler systems, fire detection apparatus, valve seals for high pressure gas container, trolley and telephone wires, and in automobile radiator finstock, iii) electrical and electronic applications such as heavy duty relays, switches, automobile distributor contacts, and solar and photocells (WHO, 2007, UNEP, 2008).

3.2.3.3. Routes of contamination

The Kahwa River catchment does not have any industry which generates Cd ions and Cd compounds in the environment. Rather, Cd pollution may arise from waste from batteries and paints that have been imported from foreign countries. In addition, incineration of municipal waste in the catchment may contribute to spread Cd dust into the environment.

Recent research showed that paint and dye may contain Cd compounds that may contaminate the environment if improperly managed (Sekomo, 2010). Furthermore, Cd pollution may be associated with fossil fuel combustion, paints, incineration of municipal waste, garage wastewater, batteries, domestic wastewater, cosmetic waste and painting workroom wastewaters (Miquel, 2001 and UNEP, 2008). Rain water may remove Cd dust from the atmosphere (Nacklé, 2003; Blinda, 2005) while contaminated wastewater from industrial and domestic activities may carry Cd into surface water. In addition, effluent from landfills and agricultural crop land can contribute to increased Cd pollution in surface water (UNEP, 2008). Once Cd enters into fresh waters, it may be adsorbed by particulate matter or precipitate in the sediment. Also, it may dissolve in water or accumulate in the bodies of aquatic organisms (UNEP, 2008). The highest Cd levels can be found in the kidney, brain and liver of organisms (WHO, 2007; Mouwerik *et al*., 2007). For example, certain aquatic species such as fish, scallops, mussels and crustaceans, living in such Cd-contaminated surface water, may ingest metal and accumulate it in the internal organs of their body (Miquel, 2001; WHO, 2007, Mouwerik *et al*., 2007). Human contamination arises through dietary intake where, owing to the large consumption of vegetables, cereals and

starchy roots, Cd can represent the greater part of daily metal intake (WHO, 2007; UNEP, 2008).

Cadmium exposure from skin contact and drinking water appears of lesser importance compared to human exposure via the diet (WHO, 2007; Mouwerik *et al*., 2007; UNEP, 2008). Most of the Cd that enters water bodies binds organic matter and then accumulate in sediment (WHO, 2007; UNEP, 2008). Secondly, it may be absorbed and accumulated in the organs of aquatic life to lower its concentration in water. However, Cd impurities in the zinc of galvanized pipes and solders in fittings can sometimes lead to increased cadmium levels in drinking-water (WHO, 2007; UNEP, 2008).

The tobacco plant naturally accumulates relatively high concentrations of Cd in its leaves (UNEP, 2008). Thus, smoking tobacco is an important source of Cd exposure. Recent research revealed that the daily intake of Cd from heavy smoking may exceed that of food ingestion (WHO, 2007; UNEP, 2008). Cigarette smoking can cause significant increases in the concentrations of Cd in the kidney, the main target organ for Cd toxicity (HWO, 2007; Mouwerik *et al*., 2007).

The research carried out in the Kahwa River catchment to assess potential pollution sources of Cd revealed that the waste generated from the activities sited in the catchment is discharged into the environment. Exposure may arise through drinking water, dietary foods and from direct contact with contaminated sediment and water from Kahwa River and the Lake Kivu. Tobacco smoking is a less important exposure route of Cd metal in Bukavu citizens as the number of smokers has decreased considerably because young people prefer drinking local beer as it is less expensive than smoking cigarettes.

3.2.3.4. Health effects of cadmium to humans and the environment

The effects of acute Cd poisoning in humans may be summarized as follows:

i) cadmium accumulation in the kidneys may lead to renal tubular dysfunction, which results in increased excretion of low molecular weight proteins in the urine, ii) a high intake of Cd can lead to disturbances in calcium metabolism and the formation of kidney stones and softening of the bones. Osteoporosis may occur in

those exposed to cadmium. In an area of Japan where soil has been contaminated with cadmium from zinc/lead mines, Itai-itai disease appeared and is still seen in women over 50 years of age. It is characterized by osteomalacia, osteoporosis, painful bone fractures and kidney dysfunction (Nacklé, 2003; Blinda, 2005, WHO; 2007; UNEP, 2008). iii) Inhalation of Cd oxide fumes results in acute pneumonitis with pulmonary oedema, which may be lethal. Long-term, high-level occupational exposure is associated with lung changes characterized by chronic obstructive disease and cancer (WHO, 2007; UNEP, 2008).

Within aquatic environments, Cd is most readily absorbed by organisms from the water in its free ionic form (Cd^{+2}). Once in the organism, it can bind to essential molecules of the cell and disturb metabolism (Nacklé, 2003; Ekpenyong and Antai, 2010). It can interact with Ca^{2+} metabolism by inhibiting calcium uptake from water (Nies, 1999). Cadmium affects the growth of plants, acting as it does on the opening of the stomata, transpiration, and photosynthesis (Belaid, 2010).

3.2.4 Characteristics of lead

3.2.4.1. Introduction

Lead is a naturally occurring metal found in small amounts in the earth's crust in a concentration varying between 8 and 20 µg/g (Tukkev *et al*., 2001; Nacklé, 2003; WHO, 2007). It is a trace metal present in water in the form of Pb^{2+} and lead compounds (Blinda, 2005). Lead is a soft, highly malleable, ductile metal and is a relatively poor conductor of electricity and is resistant to corrosion. It exists in +2 and +4 valence states and has four naturally stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb (Tukkev *et al*., 2001). Inorganic Pb compounds usually consist of Pb in the divalent state (Pb^{2+}) and it's chemistry is similar to that of the group 2 metals like beryllium, magnesium, calcium, strontium and barium (Nacklé, 2003). This similarity allows Pb ions to inhibit the absorption of calcium ions in an organism's body (Blinda, 2005; WHO, 2007). Lead has no characteristic taste or smell and its concentration in water varies between 1 and 60 µg/l (Miquel, 2001)

In the environment, Pb is usually associated with other metals such as zinc, iron, cadmium and silver (Blinda, 2005; WHO, 2007). It does not dissolve in water, but can combine with other chemicals to form lead compounds or lead salts such

as lead sulfur (PbS), lead carbonate ($PbCO_3$) or lead sulfate ($PbSO_4$) (Tukker *et al*., 2001; Nacklé, 2003). From the atmosphere, lead dust is transferred to soil, water, and vegetation via dry and wet deposition (WHO, 2010). In addition, a significant portion of lead particle emissions are of submicron size and can be transported over large distances. Larger Pb particles settle more rapidly and closer to the source of emission (Atuanya and Oseghe, 2006). With a half-life of several hundred years, new deposits of Pb, primarily atmospheric, therefore, contribute significantly to increase it's concentrations (WHO, 2007). Atmospheric deposition is the largest source of Pb in surface water. Once in water, terrestrial and aquatic organisms accumulate Pb particles from both sediment and water (Nacklé, 2003).

The accumulation of Pb in humans occurs through ingestion of food, drinking water and the inhalation of atmospheric Pb dust (Blinda, 2005). The presence of Pb in the organism is unwanted as it disturbs metabolic processes (WHO, 2007). Thus, the monitoring of Pb ions in the environment like Kahwa River is of high importance. It should help to reduce the severe effects which may be associated with the exposure to compounds contaminated by this metal.

3.2.4.2. Sources and use of lead

Lead is present in the biosphere in small amounts (WHO, 2007, Ekpo *et al*., 2008). Lead is emitted from volcano eruption as lead dust and other natural process like the degradation of rocks, soil erosion as well as forest fires (WHO, 2007). Human activities distributing Pb ions and Pb compounds in the environment include fossil fuel combustion, smelting, paints, incineration of municipal waste, the application of sewage in agriculture, runoff from urban areas, pesticides, industrial effluent, garage effluent, mining activities, storage batteries and ammunition (Blinda, 2005 and WHO, 2007). In addition, Pb pollution in surface water occurs when the metal is carried into the water through rain water run off. Effluent from mines, factories and workrooms also contribute to pollution of water (WHO, 2007, Ekpo *et al*., 2008). In countries where pipes consist of Pb, such as the Democratic Republic of the Congo, water flow may erode these pipes resulting in Pb particles in the drinking water (Blinda, 2005). Furthermore, fossil fuels have Pb compound additives such as tetraethyl lead that act as anti-knock agents. The

combustion of fossil fuels releases Pb dust into the air that returns into surface water and soil through rainwater and runoff (Monastra *et al*., 2004; Antuanya and Oseghe, 2006; WHO, 2007). In addition, the incineration of municipal solid waste, whose nature and composition is unknown, can increase the dissemination of lead particles into the environment (Blenkharn, 1995; Europian commission DG. ENV; E3, 2002). Domestic wastewater, industrial influents, urban runoff and mining effluent may form anthropogenic sources of Pb in the environment (Tukker *et al*., 2001, Monastra *et al*., 2004; WHO, 2007).

The pollution of the Kahwa River may arise naturally or from anthropogenic sources in the catchment area. The natural source of lead pollution in the Kahwa River may arise from Pb in the atmosphere as well as the degradation and erosion of soil. However, the combustion of fossil fuel in vehicles, the incineration of waste, wastewater from factories and domestic households and landfill effluents, may be the main sources of Pb in the Kahwa River.

3.2.4.3. The uses of lead

Lead is the oldest metal known to man and, since medieval times, has been used in piping, building materials, solders and paints (WHO, 2007). In more recent times, lead has been used in storage batteries, metal products, chemicals and pigment (Tukker *et al*., 2001; Sakultantimetha *et al.,* 2009). The Industrial Revolution and modern technology has increased the use of Pb in several areas of life including:

Storage batteries where Pb is mainly used in acid storage batteries;

Metal products: such as ammunition and solder, casting materials and sheet lead. It is also used in mounting of various types of equipment including air conditioning systems, heavy industrial equipment, and commercial laundry machines, additional application include cable sheathing, collapsible tubes, caulking materials and corrosive liquid containers.

Chemicals: Tetraethyl lead constitutes an anti-knock agent in fuels, and lead is used in the production of synthetic polymers;

Pigments: the use of lead in anti-corrosive and highway traffic safety paints;

Other use of lead includes automotive wheel weights, ship's ballast and various alloys, and as lead ferrite for permanent magnets in small electric motors, protect against rays in medical services, nuclear technology, piping manufacture and the manufacture of vehicle radiators (Miquel, 2001; Tukker *et al*., 2001; WHO, 2007 and Ekpo *et al*., 2008).

Lead is not applied in any human activities in the Kahwa River catchment. However, products made of Pb imported from foreign countries and the waste generated constitutes the main source of lead in the area. These include batteries, cosmetic products, paints, fossil fuel, medical waste, and piping. Once in the environment, Pb may be transported into the Kahwa River. The mismanagent of waste generated in the River catchment area plus the use of decaying Pb pipes may constitute other sources of Pb pollution of the Kahwa River.

3.2.4.5. Health risks associated with lead

Lead is toxic to all forms of life (WHO, 2007). It follows three pathways to enter in the organism bodies including ingestion, inhalation and/or surface contact (Blinda, 2005). Once the metal enters the organism, it accumulates in organs of the body such as the liver, brain, kidney, gills and bones (WHO, 2007). In humans, children between six and eleven years of age constitute the population group at greatest risk from lead exposure, mainly through ingestion. This is because their developing nervous systems are susceptible to lead-induced disruption, their intake of food is relatively high for their body weight, they are exposed to a high intake of Pb from dust, soil, and lead-containing paint due to their tendency to eat regularly and their absorption through the gut is very efficient compared to adults (Tukker *et al*., 2001; CDC, 2005). The lack of essential trace elements such as iron, calcium, and zinc and poor nourishment may increase the absorption of Pb by the human body (WHO, 2007). Inhalation poses the highest risk of exposure to environmental Pb in adults whereas inhaled airborne Pb represents a relatively small part of the body burden in children. About 30–50% of lead inhaled with particles is retained in the respiratory system and absorbed into the body (CDC, 2005; WHO 2007). In addition to environmental exposure, alcohol consumption

and tobacco smoking have been shown to contribute to human exposure to lead (WHO, 2007).

Lead affects several organs of the human body including the nervous system, bone marrow, kidneys, cardiovascular and reproductive systems. Of most concern are adverse effects of Pb on the nervous system of young children including reduction of intelligence and attention deficit, hyperactivity and behavioral abnormalities (Tukker *et al*., 2001; CDC, 2005; WHO, 2007). Many of these symptoms can be diagnosed by standardized intelligence tests. Various studies have found a highly significant association between lead exposure and the measured intelligence quotient (IQ) of school age children. Reviews of studies concluded that a 10 µg/dl increase in blood lead can be associated with a 2–2.5 point decrease in IQ with the negative impact of Pb exposure being generally greater on verbal IQ than on performance IQ (CDC, 2005). Prenatal exposure to Pb has been demonstrated to produce toxic effects in the human fetus, including reduced birth weight, disturbed mental development, spontaneous abortion, and premature birth (CDC, 2005; WHO, 2007). In addition, Pb may retard the growth and the formation of bones as it inhibits the absorption of calcium. It can increase mitotic activities and increase the incidence of chromosomal aberrations and sister chromatid exchange at blood level ranging from 22 to 89 µg/dl (Tukker *et al*., 2001). High Pb concentration, due to occupational exposure and/or accidents, results in encephalopathy, a life-threatening condition at blood lead levels of 100 to 120 µg/dl in adults and 80 to 100 µg/dl in children (CDC, 2005). The acute form of damage to the gastrointestinal tract is known as “lead colic”. The hematological effects of lead exposure are attributed to the interruption of heme biosynthesis, severely inhibiting the metabolic pathway and resulting in reduced output of hemoglobin. Reduced heme synthesis has been associated with blood levels of lead of over 20 µg/dl in adults and starting from below 10 µg/dl in children (WHO, 2007). According to the WHO, (2007), increased blood pressure and hypertension in adults is also associated with elevated blood lead levels. In addition, lead is associated with cancer where it is classified by the WHO as being one of the most important causes of cancer in humans and animals (Tukker *et al*., 2001)

3.3. Methodology

3.3.1. Study area

The current research was conducted in the Kahwa River of Bukavu sub-basin, South-Kivu province, in the East of the Democratic Republic of the Congo (DRC). The sub-basin is located at 2°29' to 2°33' latitude; 28°48' to 28°52' longitude and at an altitude between 1500m and 2194 m (Mubwebwe, 2009 and Bagalwa *et al*., 2013). It is surrounded by the Mitumba mountain chains in the South-West; Ruzizi River (connecting Lake Kivu to LakeTanganika) in the East and by Lake Kivu in the North-West (Mubwebwe, 2009; Nihoreye, 2012). The Bukavu sub-basin is characterized by a humid, tropical climate with two seasons: the dry (May to August) and the wet (September to April) seasons (Ntabugi, 2013). It is drained by the Nyakabera, Nyamuhinga, Mugaba, Tshula, Wesha, Kahwa, Mukukwe, Ruganda and Kamagema rivers (Mubwebwe, 2009). Of all of them, the Kahwa River is highly influenced by anthropogenic activities (Mubwebwe, 2009).

The Kahwa River drains 14 km^2 of the Bukavu sub-basin representing 31% of the total catchment of 45 km^2 (Mubwebwe, 2009). Several socio-economic activities including hospitals and healthcare facilities, markets, industries, laboratories, garages as well as urban areas are situated along the Kahwa River. Together, these should contribute to an increase in the pollution of river sediment and water with HMs, particularly Cd and Pb, that may have an adverse impact on the population and aquatic lives in both the river and Lake Kivu.

3.3.2. Sampling sites

In order to achieve the research objectives, samples intended for HMs analysis consisted of sediment and water and were collected from three different sites (KHW1; KHW2 and KHW3). Samples were taken at the river during the dry and rainy seasons. The criteria in selecting the sampling sites were based on the location of pollution sources and ease of accessibility to the proposed sampling sites. Sampling site three (KHW3) was located around five hundred metres from Lake Kivu and, as with the second and the first sampling sites, extended along three metres of the river. The water passing through sampling site III is expected to carry the major pollutants discharged into the river. Sampling site II (KHW2) was

located in the industrial avenue. This area hosts vehicle garages, soap factories, wash vehicles and healthcare centres. Small rivers, polluted by domestic wastewater and solid waste from healthcare facilities and workrooms in the catchment, enter the Kahwa River in the Industrial Avenue and flow through this site. Sampling site I (KHW1) is situated 100 metres from sampling site II. The wastewater and solid waste from the Bukavu central markets, slaughterhouses, garages, domestic houses and workrooms enter the Kahwa River at this point.

The Kahwa River and the sampling sites are presented in Figure 12.

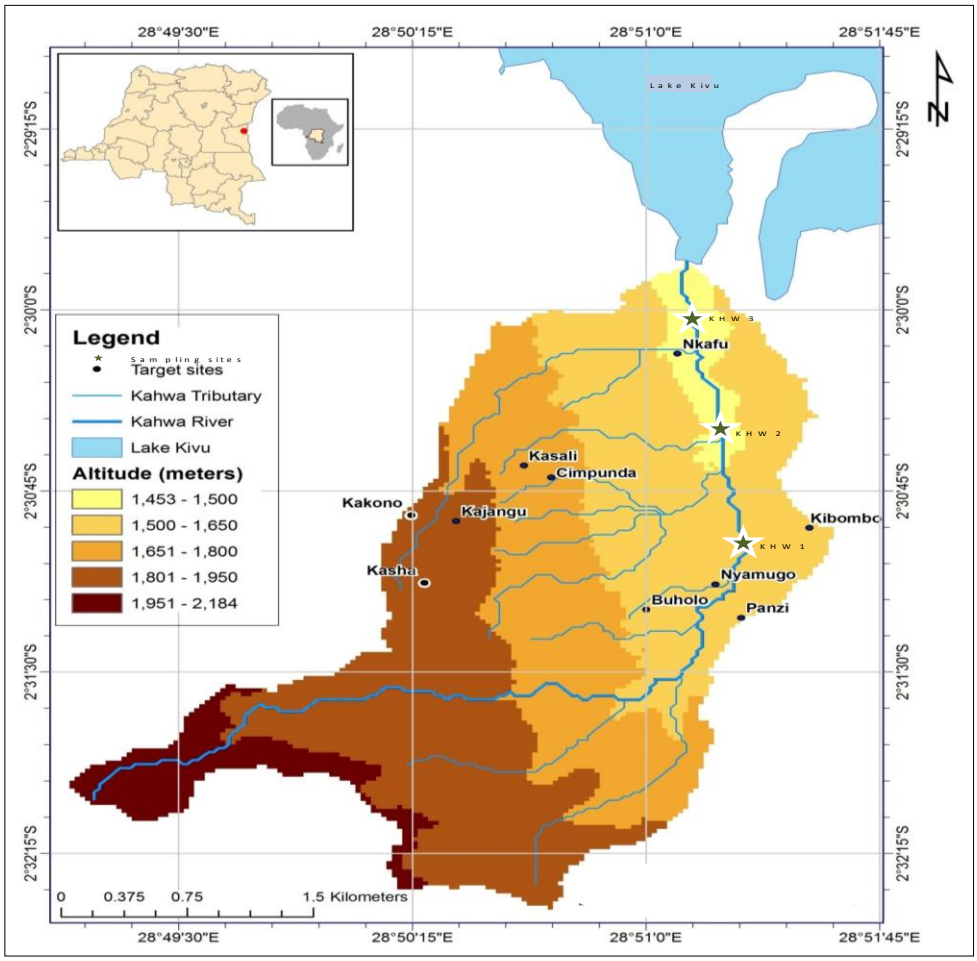

Figure 12: The Kahwa River and the sampling sites

3.3.3. Sample collection and processing

3.3.3.1. Water

Each 500 ml sample of water was collected in duplicate in plastic bottles cleaned with dilute nitric acid (10%) to avoid metal binding. The bottle, covered within aluminium layer, was rinsed three times with water from the river before being filled leaving an air space to allow adequate mixing (Reza and Singh, 2010; Sekabira *et al*., 2010). The sample was digested by mixing 95 ml of water with 5 ml of HNO_3 (65%). The mixture was heated until around 10 ml of the initial solution remained (Sekomo, 2010). The latter was transferred into a clean 100 ml flask previously rinsed three times with distilled water. At the end of the process, the flask was filled up to the mark with distilled water and filtered through a filter membrane of 0.45µm pore size (Sekomo, 2010; Reza and Singh 2010). Cadmium and lead concentrations were determined by the use of flame atomic absorption spectrometer (AAS) (Perkin Elmer model AA analyst 200) in the laboratory of the University of Rwanda at Huye.

3.3.3.2. Sediment

Plastic piping (1.3 cm in diameter and two metres long) was prepared in order to sample the sediment. It was washed with diluted nitric acid (10%) and rinsed three times with the water of the river before being used. In the process, the pipe was pushed into the sediment until the required quantity of sediment was obtained. It was collected in duplicate and stored in the bottles covered within aluminium layer before being dried in an oven at 103^oC for 24 hrs. The dried sediment was ground and sieved through a sieve of 1 mm pore size. A mass of 1,250 g was digested in hydrochloric acid (HCl) (37%), nitric acid (HNO_3) (65%) and hydrogen peroxide (H_2O_2) (30%) for two days as described by Barifaijo *et al*., (2009) and Sekomo *et al*., (2010). Thereafter, the mixture was poured into a 100 ml flask and stored at low temperature to cool down. Then, the flask was filled to the 100 ml mark with distilled water and stored overnight to allow complete settling of metals. The mixture was filtered through a filter membrane of 0.45µm pore size.

3.3.4. Sample analysis

The determination of the Cd and Pb concentrations in sediment and water was performed using a flame AAS (Perkin Elmer model AA analyst 200) in the Laboratory of Science of University of Rwanda at Huye.

3.3.4.1. Standard solution

The standard solution was prepared from the stock solution (1000 mg/l). From the stock solution, the concentration of cadmium (2 mg/l) and lead (15 mg/l) was prepared according to the following fundamental volumetric formula (Perken Elmer, 2002), respectively:

$$C_1 \times V_1 = C_2 \times V_2$$

Where,

C_1: Concentration of the stock solution;

V_1: Volume of the stock solution;

C_2: Concentration limit of the heavy metal;

V_2: Volume of the limit solution.

A blank solution was used that consisted of the chemicals and distilled water used to digest the sample prior to analysis.

3.3.4.2. Detection of heavy metals

Analysis of HMs in the samples of sediment and water was performed using AAS. In the process, the AAS was switched on to allow aspiration of the blank solution into the capillaries of the apparatus. This was followed by aspiration of the standard solution prepared for Cd or Pb. The analysis of the blank solution and standard solutions allowed the AAS to draw the calibration curve for each metal. Thereafter, the capillary column was washed by aspirating distilled water followed by the aspiration of the blank solution. This step was followed by the aspiration and analysis of filtered sample collected in the apparatus to determine Cd and Pb concentrations.

3.3.5. Data analysis

The data obtained in the research were analyzed by the statistical software ‘’xlstat’’. The Parametric test (t-test) was applied to assess differences in Cd and Pb concentration between sampling sites. For this analysis, as river water flowed from

sampling site I to site III, a comparison was performed in the following order: sampling site I was compared to II; then site II was compared to site III and then site I to site III. In addition, the sampling sites were compared for the concentration of the metals in water and sediment. Thus, site I (water) was compared to site I (sediment) and this was repeated for sites II and III.

3.4. Results and discussion

3.4.1. Results

3.4.1.1. Water

The research carried out on water of the Kahwa River in the wet and dry seasons showed that it was polluted by Cd and Pb (Fig. 13). The concentrations obtained for each metal were higher than the limit set by the WHO for drinking water. These limits are 0.003 mg/l for Cd and 0.01 mg/l for Pb (Sekomo, 2010; Wogu and Okaka, 2011). It was observed that the concentration of Pb was higher than that of the Cd in all the sampling sites (Fig. 13). This could result from the river receiving much more Pb than Cd from the environment. The comparison between sampling sites as to the concentration of Cd and Pb in water did not reveal a significant difference ($p>0.05$). This may be explained by the fact that the activities sited along the river are likely to be similar and, thus, generate the same type of waste which is discharged in the Kahwa River (Fig. 5 and 7).

The mean concentration of Cd and Pb in water in the wet and dry seasons is presented in Fig. 13. These results indicate that in comparison to the WHO limits of 0,003 mg/l for Cd and 0,01 mg/l for Pb in water, the Kahwa River contains approximately six times the accepted level of Cd and approximately 30 times the accepted level of Pb.

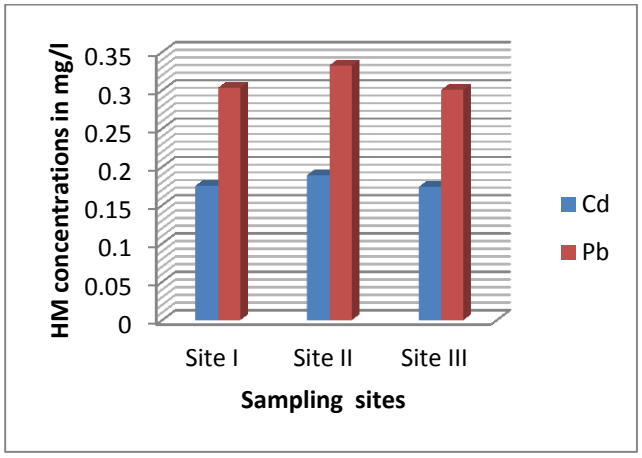

Figure 13: Mean value of Pb and Cd concentration in water

3.4.1.2. Sediment

The assessment of Cd and Pb concentration in sediment collected in the Kahwa River showed that it is polluted by Cd and Pb. The concentration measured

was higher than the limit set for sediment by Macdonald and Ingersoll (2000) of 99 x 10^{-4} mg/g for Cd and 358 x 10^{-3} mg/g for Pb as cited by Sekomo (2010) and Kaki *et al*. (2011). The concentration of Cd was lower than that of the Pb (fig.14) at all the sampling sites. This could be linked to activities generating significant amounts of lead and its release into the Kahwa River catchment. A comparison between sampling sites as to the concentration of Cd and Pb in sediment did not reveal a significant difference ($p>0.05$) with the exception of Cd at sites II and III and I and III. This might be explained by sewage being thrown into the Kahwa River and it tributaries. However, a comparison between sampling sites as to the concentration of Cd and Pb in water and sediment showed a significant difference $p<0.05$). This might be justified by the fact that the sediment accumulates most metal discharged into the river (Reza and Singh, 2010; Kaki *et al*., 2011)

The Average Pb and Cd concentration in sediment in the wet and dry seasons is presented in figure 14. These results indicate that in comparison to the accepted limits of 0,0099 mg/g for Cd and 0,358 mg/g for Pb in sediment, the Kahwa River sediment contains approximately 5000 times the accepted level of Cd and approximately 600 times the accepted level of Pb.

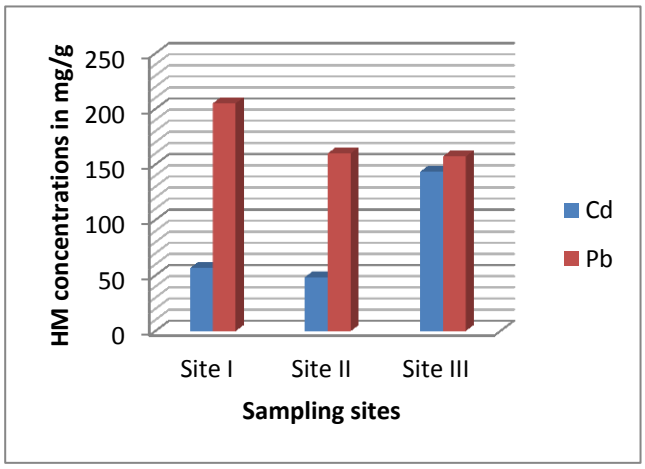

Figure 14: Average Pb and Cd concentration in sediment.

3.4.2. Discussion

The presense of heavy metals such as Cd and Pb in the environment are of concern as they are among the most toxic of metals (WHO, 2007). The research carried out to assess the concentration of Cd and Pb in the sediment and water of

the Kahwa River in the dry and wet seasons showed that the river was extensively polluted by both Cd and Pb. The concentration recorded for Cd was six times higher than the limits set by the WHO (2008) for drinking water and 30 times the limit for Pb. In the case of the river sediment, the concentration of Cd was 5000 times the accepted limit for sediment as described by Macdonald and Ingersoll (2000) as cited by Sekomo (2010); Kaki *et al*. (2011) and 600 times the accepted limit for Pb in the sediment. These results correlate to those found by Sekomo (2010) in Nyabugogo River. Pollution with HMs in the Kahwa River might be explained by anthropogenic activities and population growth in the river catchment which include garages, healthcares, factories and markets. Sekomo (2010) and Adelekan and Abegunde (2011) reported that wastewater generated in the garages is the major source of Cd and Pb in the environment. According to the WHO (2007); Samir and Ibrahom (2008) and Akan *et al*. (2010), domestic and municipal effluents discharged in the river lead to HMs contamination of the river water and sediment.

The research performed in the Kahwa River catchment revealed that the waste generated in several activities performed in the river catchment is either incinerated or discharged into the canals, small rivers and the ground, all potentially contributing to pollution by HMs of the Kahwa River. The Kahwa River catchment is home to motor bikes and vehicles that use fossil fuel and the combustion of fossil fuel may release Cd and Pb in the environment (Atuanyi and Oseghe, 2006). The incineration of solid municipal waste may spread Cd and Pb in the environment and these metals may return through runoff and deposition (Nacklé, 2003). In addition, pipes distributing potable water throughout Bukavu Town consist of Pb and these may corrode leading to lead particles in the water (Blinda, 2005; WHO, 2007).

The concentration of Pb in sediment and water was higher than that of the Cd in all the sampling sites. These results were reported by Sekomo (2010) in the Nyabugogo wetland and by Mutuku *et al*. (2014) in the wetland of the Lake Victoria. This may be linked to activities generating much more Pb than Cd (Blinda, 2005, Sekomo, 2010) but also to strong absorption and bioaccumulation of Cd (Samir and Ibrahim, 2008). The comparison between sampling sites in the

concentration of Cd and Pb in water and sediment revealed a significant difference ($p<0.05$) (fig. 13 and 14). This observation correlates with results reported by Sekomo *et al*. (2009) obtained in the Nyabugogo River.

Zakir and Shikazono (2011) reported that metals discharged in the river accumulate in sediment in various forms which include ion exchange, adsorption, precipitation and complexation. According to Akan *et al*. (2010) and Kaki *et al*. (2011), HMs polluting aquatic ecosystems accumulates in the sediment making it the “chemical archives” of heavy metals accumulations. This may explain why the concentration of the HMs was relatively much higher in sediment than in water in all the sampling sites.

3.5. Conclusion

The assessment of HMs concentration in the urban rivers has become a research concern worldwide to reduce the health risk to human and aquatic life (Reza and Singh, 2010; Akan *et al*., 2010).

The results obtained in the investigation revealed that the sediment and water were polluted by Cd and Pb. The concentration of these metals in the sampling sites was higher than the limits set by the WHO for drinking water and sediment (Sekomo, 2010; Kaki *et al*., 2011). The contamination of the Kahwa River may be explained by the sewage discharged into it and its tributaries from point and non-point sources. The comparison between sampling sites didn’t show a significant difference ($p>0.05$) in general. This may be explained by a consistent, fairly similar production of the waste produced by humans in the catchment area.

The pollution of the Kahwa River by Cd and Pb should have a significant impact on human beings and aquatic lives through ingestion of the metals, via the food chain and via contact with contaminated sediment and water (Blinda, 2005). Particular attention should be focused on the removing of the metals in order to restore water quality in the river. In this regard, decisions should be taken to collect and/or to prevent the discharge of untreated waste in the Kahwa River in order to protect the population and the biodiversity.

CHAPTER 4: ASSESSMENT OF PATHOGENIC BACTERIA IN THE KAHWA RIVER

4.1. Introduction

Life started in water and evolved in it before being extended onto land. And life still depends on water (Hamid *et al*., 2007). Apart from being essential for life, water directly contributes to human activities such as agriculture, industry, and recreational activities. After being used, it is returned to the environment and is often contaminated with chemicals and organisms that alter its quality (Corcoran *et al*., 2010; UN-Water, 2011). Water has become, therefore, a source of disease which places at risk the health of humans and animals (Katarina and Payment, 2005).

Recent research carried out on water quality revealed that waterborne diseases caused by *Vibrio cholerae* (serotypes O1 and O139), *Salmonella typhi* and *paratyph*i, *Shigella dysenteriae* and *Escherischia coli* (serotype O148, O157 and O124) were responsible for epidemic diseases mainly in developing countries due to poor hygiene and sanitation (Katarina and Payment, 2005; Hamid *et al*., 2007). Up to 80% of all diseases in the world are caused by inadequate sanitation, polluted water and water scarcity (Battu and Reddy, 2009; Corcoran *et al*., 2010; Manjula *et al*., 2011; Salomon *et al*., 2011). According to Salomon *et al*. (2011), approximately three out of five persons in developing countries do not have access to safe drinking water. In addition, waste may be discharged into surface water, leading to the contamination of water with enteric parasites and heavy metals (Nageswaran *et al*., 2012 and Mutiku *et al*., 2014). Bacteria evolving in such environments can develop resistance mechanisms to withstand metal pollution that may contribute to the development of resistance to antibiotics (Spain and Alm, 2003; Nageswaran *et al*., 2012). Fortunately, bacteria that are resistant to heavy metals and have the capacity to survive in environments with high HMs concentrations can play an important role in bioremediation where they can facilitate the removal of HMs (Anyanwu and Ugwu, 2010).

Economic development and population growth in the Kahwa River catchment has resulted in an increase in the production of waste which is followed by the pollution of the sediment and water of this river. The pollution of the Kahwa

River is explained by the fact that the waste generated in the catchment is discharged into the environment without treatment. Most canals and tributaries in the catchment flow into the Kahwa River and contribute towards its pollution with microbes and HMs.

Pollution related to HMs and pathogenic bacterial adaptation in the Kahwa River has never been investigated and other data on this river are still scarce. The current study aims to isolate microbes from both the sediment and the water of the Kahwa River and to perform susceptibility tests in regard to resistance to antibiotics and tolerance to heavy metals, particularly Cd and Pb.

4.2. Review of literature

4.2.1 Enteric bacteria

Bacteria constitute a group of organisms that can only be seen with the aid of microscopes (Tortora *et al*., 2001). Some of them, the microflora, are harmless and live naturally in the gut of humans and warm-blooded animals and are called indicator microbes as their presence in water indicates faecal pollution (Melita *et al*., 2003; Carlender, 2006). However, pathogenic bacteria can cause diseases in humans and the biodiversity when they are ingested and/or through direct contact (Tortora *et al*., 2001; Pappas *et al*., 2008; Lotter, 2010). The indicator and pathogenic bacteria concerned in this research study are described in the following sections.

4.2.1.1. Indicator Bacteria

i. Definition

Indicator bacteria are microorganisms living naturally in symbiosis in the gut of humans and warm blooded animals. Under normal conditions, they do not grow or multiply outside the host organism (Melita *et al*., 2003; Lotter, 2010). Their presence in water indicates pollution attributed to human and animal faeces.

Indicator bacteria are easily analyzed by standard bacteriological methods and provide information on the extent of faecal pollution of water (Carlender, 2006; Lotter, 2010). These bacteria consist of the following groups: total coliform, faecal coliform and faecal enterococci (Melita *et al*., 2003) which are described in the following section.

ii. Description of indicator bacteria

Coliforms or total coliforms are defined as being aerobic or facultative anaerobic bacteria, Gram negatives that ferment lactose and produce gas within 48 hrs (Hamid *et al*., 2007; Borrego and Figueras, 2010). They do not form endospores, have a rod shape and possess β-galactosidase (Melita *et al*., 2003). Total coliforms are classified as indicators of faecal contamination of water because they were considered to inhabit, exclusively, the gut of humans and other warmblooded animals (Carlender, 2006; Figueras and Borrego, 2010). However, the ability of these coliforms to grow in natural waters, the lack of correlation between the number of coliforms and those of pathogens has led to their being unsuitable faecal indicators of water pollution (Melita *et al*., 2003; Carlender, 2006; Figueras and Borrego, 2010). In addition, recent research revealed the presence of coliforms in drinking water distribution systems in the absence of faecal contamination (Carlender, 2006; Figueras and Borrego, 2010). Nowadays, total coliform counts are associated with monitoring of sewage and water treatment plants and should be absent from adequately treated plant effluents (Figueras and Borrego, 2010). The presence of total coliforms in distribution system, due to inadequate treatment, could be due to laboratory cross contamination or to a failure to maintain adequate disinfection from sewage works (Carlender, 2006; Figueras and Borrego, 2010).

Faecal or thermotolerant coliforms are a group of indicator bacteria that fulfill the criteria used to define total coliform plus the additional information that they grow and ferment lactose with the production of acid at 44^{o}C and 45^{o}C (Carlender, 2006; Figueras and Borrego, 2010). For this reason, the name ‘’thermotolerant coliforms’’ was given to them (Carlender, 2006; Figueras and Borrego, 2010). Some thermotolerant coliform bacteria that conform to the definition above belong to the genus *Klebsiella* and have been isolated from environmental samples in the apparent absence of faecal pollution (Carlender, 2006). Similarly, other members of the faecal coliform group, *Escherishia coli*, have been detected in unpolluted samples. For example, in tropical and subtropical climates, thermotolerant coliforms have been isolated in water without any correlation with human and animal faeces (Carlender, 2006). Thus, the

occurrence of faecal coliform in tropical water does not necessarily suggest faecal contamination (Carlender, 2006; Lotter, 2010).

Escherichia coli (*E. coli*) is a member of the faecal coliform group considered as being a more specific indicator of water faecal contamination (Melita *et al*., 2003; Figueras and Borrego, 2010). It conforms to taxonomic, functional identification criteria of coliforms and it is enzymatically distinguished by the lack of urease and the presence of β-galactosidase and β-glucuronidase (Carlender, 2006; Figueras and Borrego, 2010). One of the disadvantages associated with *E. coli* as an indicator of faecal pollution is that it has been isolated in pristine tropical rain forest and in the soil as well (Carlender, 2006; Figueras and Borrego, 2010). It grows at 37^{o}C to 45^{o}C and ferments lactose followed by the production of acid and gas (Melita et al., 2003; Lotter, 2010). In addition, it appears to survive for a short period in an aquatic environment outside the gut of humans and other warm-blooded animals (Melita *et al*., 2003; Carlender, 2006). In spite of these disadvantages displayed by *E. coli*, it has been selected by the WHO as being the faecal indicator of choice for drinking water (Figueras and Borrego, 2010).

Several countries include this bacterium in their regulations as the best indicator of faecal pollution in water and food (Melita *et al*., 2003; Carlender, 2006; Figueras and Borrego, 2010). Although the genus consists of harmless species, some strains like *E. coli* (O157:H7) have been linked with disease outbreaks associated with contaminated drinking water or food resulting in haemorrhagic colitis, gastroenteritis and kidney failure (Thi Thu Hao Van, 2007).

Faecal e*nterococc*i, *Streptococci* or Intestinal *enterococci* have received widespread acceptance as a useful indicator of faecal pollution of water since: i) they occur in higher number in the aquatic environment compared to pathogens (around 10^6 per 100 ml of raw domestic wastewater), ii) they are always present, especially, in the faeces of human and other warm-blooded animals, iii) they are unable to multiply in the environment and iv) their die-off in water is less rapid than that of pathogenic bacteria v) they are easily detected by simple standard methods and they can resist environmental stresses for a long time (Lotter, 2010; Figueras and Borrego, 2010). Faecal *streptococci* are gram positive, catalase negative and facultative anaerobic bacteria and are always

associated with faecal pollution of human and warm blooded animals (Malita *et al*., 2003).

4.2.1.2. Pathogenic bacteria

i. *Shigella* spp

Organism and origin: Members of *Shigella* spp. are gram negative, non-motile bacteria belonging to the family of enterobacteriacae (Cheryl *et al*., 2002). The genus consists of four species classified on the basis of biochemical and serological characteristics including *Shigella dysenteriae* (*S. dysenteriae)*, *S. flexneri*, *S. boydii* and *S. sonnei,* respectively, named the A, B, C and D groups, often associated with shigellosis (Sack *et al*., 2001; WHO, 2005). The first three species (A, B and C), are further divided into serotypes increasing, thus, the number of pathogenic species within the genus. Of these serotypes, *S. dysenteriae* serotype1 (*S. dysenteriae1*) (Shiga bacillus) has been awarded special attention because it is more likely to cause severe disease, to spread in epidemics, to be particularly resistant to antibiotics and to produce shiga toxin (Sack *et al*., 2001; Cheryl *et al*., 2002).

Reservoirs of *Shigella* spp: *Shigella* spp. have as reservoirs humans and primates in captivity from where they can spread into the aquatic ecosystems (Sack *et al*., 2001). The minimal infection dose is less than 200 cells which may facilitate transmission. *Shigella* is mainly found in highly crowded facilities such as day care centres, military camps and prisons characterized by poor sanitation and poor hygiene (Sack *et al*., 2001; Cheryl, 2002).

Geographical distribution of *Shigella* spp: This varies within *Shigella* species. For example, *S. flexineriae* and *S. dysenteriae* are most commonly isolated in developing countries, *S. dysenteriae* and *S. boydi* infection also occurs in less developed countries. However, in developed countries, *S. sonnei* is predominant (Sack *et al*., 2001). In recent years, *Shigella* spp have caused dysenteric epidemics in Central America, south Asia and in central Africa where they started in 1979 in central Africa, particularly in the east of the DRC, Rwanda and Burundi. In the beginning of 1990, the epidemic headed southwards to affect first Zambia, Mozambique, Zimbabwe and later South Africa (Cheryl *et al*., 2002).

It is estimated that *Shigella* spp causes eighty million cases of bloody diarrhea and seven hundred thousand deaths each year. Ninety nine percent of these deaths occur in developing countries where the majority of infections (70%) and deaths (60%) involve children less than five years of age (WHO, 2005; Christopher *et al*., 2010).

Epidemiology and transmission of *Shigella* spp: *Shigella* spp are transmitted by contaminated food, drinking water and from person to person. Flies (*Musca domestica*) may also facilitate *Shigella* transmission to human materials (WHO, 2005). Recent research has shown that *Shigella* spp were responsible for a high fatality rate among hospitalised children less than five years of age, particularly if signs of malnutrition were observed in these patients. During epidemics, mortality rates as high as 3.9% in children under the age of one year and 19.3% in infants less than four months of age have been reported. In Central Africa, a high mortality rate was also observed in young adults (Sack *et al*., 2001).

Symptoms of shigellosis: Dysentery associated with shigellosis infection is characterized by fever, diarrhea containing blood with or without mucous accompanied by abdominal cramps and ineffectual and painful straining during the production of stool or urine (Sack *et al*., 2001; Christopher *et al*., 2010). Severe complications in infected person include metabolic abnormalities, sepsis, convulsions, prolapsed rectum, toxic mega-colon, intestinal perforation and haemolytic-uraemic syndrome (Christopher *et al*., 2010).

Therapy for shigellosis: Appropriate therapy for shigellosis aims to reduce the risk of complications and deaths related to infection (Sack *et al*., 2001). The antimicrobial agents recommended in the WHO guideline include ampicillin (Amp), sulfamethaxazole-trimethroprime, nalidixic acid, ciprofloxacin (Cip); norfloxacin, and enoxacin (Cheryl *et al*, 2002). The misuse of antibiotics and inappropriate waste disposal from human activities has increased antibiotic resistance in *Shigella* spp in developing countries and this may increase the rate of mortality and morbidity resulting from this infection (Sack *et al*., 2001).

ii. *Salmonella* spp

Description of the genus: *Salmonella* spp are facultative, anaerobic bacteria that are gram negative rods, non-capsular with peritrichous flagella and are not spore formers. They can ferment glucose and lactose (Lepage, 2009). Formally, three species of *Salmonella* existed, namely *Salmonella enterica (S. enterica), S. bongori* and *S. subterranea* (Lepage, 2009 and David, 2010). The species *S. enterica* includes by itself six subspecies: *S. enterica* subsp *enterica*, *S. enterica* subspecies *salamae*; *S. enterica* subspecies *arizonae*; *S. enterica* subspecies *diarizonae*; *S. enterica* subspecies *houtenae and* S. enterica subspecies *indica,* (Pouget, 2006; Lepage, 2009; David, 2010). *Salmonella* spp continue to increase in number and currently there are more than 2500 serovars (Thi Thu Hao Van, 2007). The genus *Salmonella* appeared to be limited to the digestive tract. Recent research revealed its presence in faecally contaminated environments such as water, sediment and food (Võ Thi Tră An, 2007; Thi Thu Hao Van, 2007). Most serotypes of *Salmonella* have a broad host spectrum but some serotypes infect a single host species. These include *S. typhi* and *S. paratyphi* in humans, *S. dublin* in cattle and *S. choleraesius* in pigs (Thi Thu Hao Van, 2007).

Epidemiology and transmission: *Salmonella* spp are responsible for a higher proportion of deaths than any other pathogenic bacteria in many countries and the transmission is by faecal-oral route (Lepage, 2009). Although all *Salmonella* spp are pathogens, the majority of human infections are caused by *S. typhi* and *S. paratyphi* (Lepage, 2009). Typhoid fever is a serious infectious disease caused by *S. typhi* and it constitutes an important public health threat in developing countries. The disease is characterized by prolonged fever, growth of bacteria in cells of the reticuloendothelial system, and a significant inflammation of the lymphoid organs of the small intestine (Thi Thu Hao Van, 2007). The consumption of contaminated food or water with *S. typhi* can result in typhoid fever (Lepage, 2009). Paratyphoid serotypes consist of *S. paratyphi* A, *S. paratyphi* B and *S. paratyphi* C. that cause a similar syndrome, typhoid fever syndrome to that caused by *S. typhi* (Lepage, 2009).

Studies on the annual global burden of typhoid fever reported that, worldwide, the cases of salmonellosis were estimated at 1.3 billion cases and 3 million deaths (David, 2010). Factors involved in the exposure to *Salmonella* infection include the patient's age, immunity and nutritional status and socio-economic factors (Lepage, 2009). Contaminated water, fish, fruits, vegetable, eggs and milk are the main sources of transmission of *Salmonella* spp in humans (Thi Thu Hao Van, 2007). Furthermore, *Salmonella* spp were reported in soil, fertilizer and drinking water (David, 2010). The pollution of sediment and water in the Kahwa River can occur from such non-point sources.

Therapy for salmonellosis: Salmonellosis is an intestinal infection characterized by diarrhea, vomiting, chills and headache followed by dehydration (Thi Thu Hao Van, 2007). The treatment of thyphoid fever requires fluid, electrolyte replacement and antibiotics. Drugs such as chloramphenicol (C), Amp, Cip and amoxicillin and trimethroprim-sulfametroxazole are mostly recommended (Perilla *et al*., 2002).

iii. *Vibrio* spp

Description of the genus: *Vibrio* spp are gram negative, facultative anaerobic, curved and rod-shaped bacteria with flagella (Environment Agency, 2002). The natural habitat of *Vibrio* is an aquatic environment where it can survive at maximum and minimum temperatures of 43°C and 5°C, respectively. *Vibrio* spp are sensitive to acid media and they can grow at a pH slightly above neutrality, i.e. pH 7.5 to 8.5 (Thi Thu Hao Van, 2007). The genus *Vibrio* includes the following species: *Vibrio cholerae (V. cholerae)*, *V. vulnificus* and *V. parahaemolyticus.* Of these species, *V. cholerae* causes cholera and includes nearly 200 serogroups based on O antigenic structures. *Vibrio cholerae* O1 and *V. cholerae* O139 are associated with epidemics and clinical syndromes of cholera (Cheryl *et al*., 2002).

Epidemiology and transmission: *Vibrio* spp live in the intestine of infected individuals. It is released into water bodies through faeces (Environment Agency, 2002). Infection with *Vibrio* may occur through ingestion of contaminated water and food (Thi Thu Hao Van, 2007). The symptoms related to cholera include profuse watery diarrhea, vomiting, and muscle cramps (Environment Agency,

2002; Cheryl *et al*., 2002). Severe cholera is characterized by "rice water" stools, loss of ten percent or more of body weight, loss of normal skin turgor, dry mucous membranes, sunken eyes, lethargy, anuria, weak pulse, altered consciousness, and circulatory collapse. Diarrhoeal fluid loss may result in profound hypokalemia, metabolic acidosis (from bicarbonate loss) and renal failure. Severe infections may result in death (Cherly *et al*., 2002). Pathogenic bacteria live naturally in the gut of humans and other warm blooded animals. When these organisms are released into an external environment, most of them die and only a few survive. The mechanisms allowing adaptative survival of the bacteria in aquatic environments include mutation and transfer of resistance genes among microbes (Giger *et al*., 2003; Spengler, 2006; Džidič *et al*., 2008; Blair, 2010).

4.2.2. Antibiotics

4.2.2.1 Introduction

Antibiotics are chemical substances naturally produced by microorganisms and fungi (Schmieder, 2012) that either kill or inhibit the growth of pathogenic bacteria and other organisms (Kohanski *et al*., 2007; Džidič *et al*., 2008). They function by attacking the following targets: protein synthesis, nucleic acid replication and repair and cell wall biosynthesis enzyme and substrates (Thi Thu Hao Van, 2007; Džidič *et al*., 2008; Kümmerer, 2009; Blair, 2010; Schmieder, 2012). Antibiotics have been used to combat infectious diseases worldwide (Manisha *et al*., 2011) and their discovery provided an expectation that diseases should disappear (Džidič *et al*., 2008). Unfortunately, microbes have developed resistance to antibiotics, thus, compromising treatment of diseases (Blair, 2010; Schmieder, 2012).

Many factors influence microbial resistance to antibiotics including: the excessive use and the misuse of drugs; the nutritive and theurapeutic antibiotic treatment of farm animals (Thi Thu Hao Van, 2007; Džidič *et al*., 2008; Kümmerer, 2009) and the use of antibiotics in agrictural crop lands in the absence of acute plant infection (Heather *et al*., 2010), and the pollution of ecosystems by HMs (Spain and Alm, 2003; Nasrazadani *et al*., 2011). Regarding the first point above, antibiotics consumed by patients are not totally absorbed in the body, the majority

being released into the environment through the urine and faeces (Abu and Egenonu, 2008; Thi Thu Hao Van, 2007). The release of faeces in water may increase microbial antibiotic resistance (Džidič *et al*., 2008; Kümmerer, 2009). Furthermore, the misuse of antibiotics in human medicine has increased the spread of antibiotic resistance among environmental bacteria. The causes of this misuse are likely linked to incorrect drug selection, dose and duration of the antibiotic treatment (Yah and Eghafona, 2008). The pharmaceutical industry plays an important role in excessive and inappropriate antibiotic use due to marketing strategies to promote the use of broad-spectrum drugs (Võ Thi Tră An, 2007). Moreover, expensive recuperation spent in hospitals in many developing countries has led to patients preferring outdoor antibiotic therapy over hospital therapy and such therapy is often stopped when the patient feels better (Võ Thi Tră An, 2007).

Bacteria evolving in environments polluted with HMs and antibiotics develop resistance to antibiotics (Mutuku *et al*., 2014). There are four major mechanisms of antibiotic resistance which include: (i) inactivation of antibiotics by detoxifying enzymes or antibiotic modification by extracellular enzymes (Beta-lactamase, Chloramphenicol acetyltransferase), (ii) alteration or modification of the antibiotic target that reduces its binding capacity (mutation of the key binding elements such as ribosomal RNA), (iii) reduction of the intracellular antibiotic concentration by decreasing permeability and/or increasing the activities of efflux pumps such as AcrAB and TolC) and metabolic bypass (Mudryk, 2001; Blair, 2010; Manisha *et al*., 2011). All of these processes occur in aquatic environments (Mudryk, 2002). The resistance of bacteria to antibiotics reduces the efficacy of the drug and, thus, can compromise the health of patients following their infection with antibiotic-resistant bacteria (ARB) and the dissemination of antibiotic resistant genes (ARG) among microbial populations in biofilms (Abu and Egenonu, 2008; Thi Thu Hao Van, 2007).

Antibiotics are used by local citizens mainly without medical prescription. This practice results from excessive poverty and expensive treatment costs associated with hospitalization, medical consultation and extended time spent in a hospital, such as happens in Bukavu Town. The citizens prefer to pay directly for

drugs in pharmacies and they stop taking them when they feel well, without necessarily completing the prescribed course of drugs.

The research carried out in the Kahwa River catchment revealed that the waste from human activities is discharged into the river, canals and onto the ground. The disposal of human waste, urban waste contaminated by HMs and antibiotics together with the excessive and the misuse of antibiotics by the population can combine to increase antibiotic resistance in environmental bacteria. The current study related to antibiotic resistance shown by bacteria isolated from the Kahwa River is novel. The action of antibiotics and the form of resistance in bacteria is discussed below.

4.2.2.2. Antibiotic action

Antibiotics act on bacteria and should not have any effect on the eukaryotic host cells (Yoneyama and Katsumata, 1996, Thi Thu Hao Van, 2007). The effects of these drugs are either bacteriostatic or bactericidal as they can prevent the growth of, or kill, bacteria (Džidič *et al*., 2008, Schmieder, 2012). Antibiotics act on bacteria in several ways including i) the inhibition of cell wall synthesis, ii) the inhibition of protein synthesis and iii) the inhibition of nucleic acid synthesis (Kohanski *et al*., 2007; Džidič *et al*., 2008, Kohanski *et al*., 2010; Schmieder, 2012).

i. Inhibition of cell wall synthesis

Bacterial cells, evolving in an aquatic environment, are subject to water imbalance, leading to high intracellular pressure.They are protected against this pressure by tough peptidoglycan layers (Yoneyama and Katsumata, 1996). The latter consists of a network of sugars or carbohydrates, glycans, and peptides linked together by covalent bonds (Yoneyama and Katsumata, 1996; Blair, 2010). The degree of peptidoglycan cross-linking correlates with the structural integrity of the cell wall and the capacity to withstand host environmental conditions (Blair, 2010). The maintenance of peptidoglycan is accomplished by the activities of enzymes such as transglycosylase and Penicillin Binding Proteins (PBPs), also called transpeptidases (Yoneyama and Katsumata,1996).

β-lactams such as penicillins, carbapenems and cephalosporins inhibit synthesis of the bacterial cell wall where they interfere with the enzymes required for the synthesis of the peptidoglycan layer (fig. 15) (Kohanski *et al*., 2010). However, the glucoptides such as vancomycin and teicoplanin interfere with the cell wall synthesis (Blair, 2010). They do so by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross linking steps required for stable cell wall synthesis (Kahanski *et al*., 2007; Džidič *et al*., 2008; Kohanski *et al*., 2010). Glycopeptides can inhibit peptidoglycan maturation and may reduce the mechanical strength of the cell (Yoneyama and Katsumata, 1996; Tonover, 2006; Džidič *et al*., 2008; Blair, 2010).

The treatment of bacterial infection with cell wall synthesis inhibitor antibiotics can result in changes to bacterial cell shape and size, induction of cell stress responses and, ultimately cell death (Džidič *et al*., 2008, Kahanski *et al*., 2010).

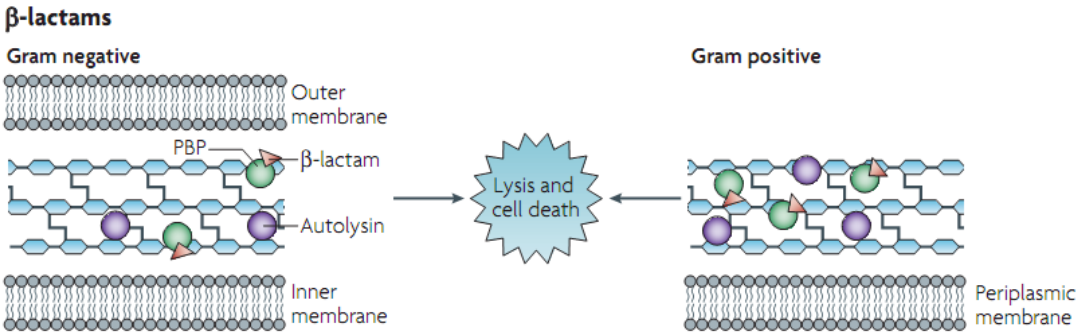

Figure 15: Bacterial cell wall inhibition by beta-lactam antibiotics (Adapted from Kohanski et al., 2010)

ii. Inhibition of protein synthesis

Biosynthesis of proteins is carried out in ribosomes, a structure which consists of about two-thirds RNA and one-third protein (Yoneyama and Katsumata, 1996; Džidič *et al*., 2008). The ribosome of bacteria consists of two ribonucleoprotein subunits, the 50S and 30S subunits, which assemble in the cytoplasm during the initiation step of translation. This follows transcription, the synthesis of messenger RNA (mRNA) (Yoneyama and Katsumata, 1996). The

mRNA translation process occurs over three important phases: initiation, elongation and termination (Kahanski *et al*., 2010). Due to these several steps, an antibiotic may inhibit one of the protein biosynthesis steps and, therefore, all processes involving bacterial protein synthesis (Yoneyama and Katsumata, 1996; Džidič *et al*., 2008). The class of antibiotics involved in the inhibition of bacterial protein biosynthesis is divided into two groups: the 50S inhibitors and the 30S inhibitors. The 50S ribosome inhibitors include macrolides, clindamycin, quinupristin, chloramphenicol, dalfopristin and linezolid whereas aminoglycosides and tetracyclines inhibit the 30S ribosome subunit (Kohanski *et al*., 2OO7; Kohanski *et al*., 2010). Bacterial ribosomes differ from their homologue in eukaryotic cells in structure and medicine has taken advantage of these differences to use antibiotics to selectively inhibit bacterial growth (Tonover, 2006). The protein biosynthesis inhibition in bacteria is presented in Figure 16.

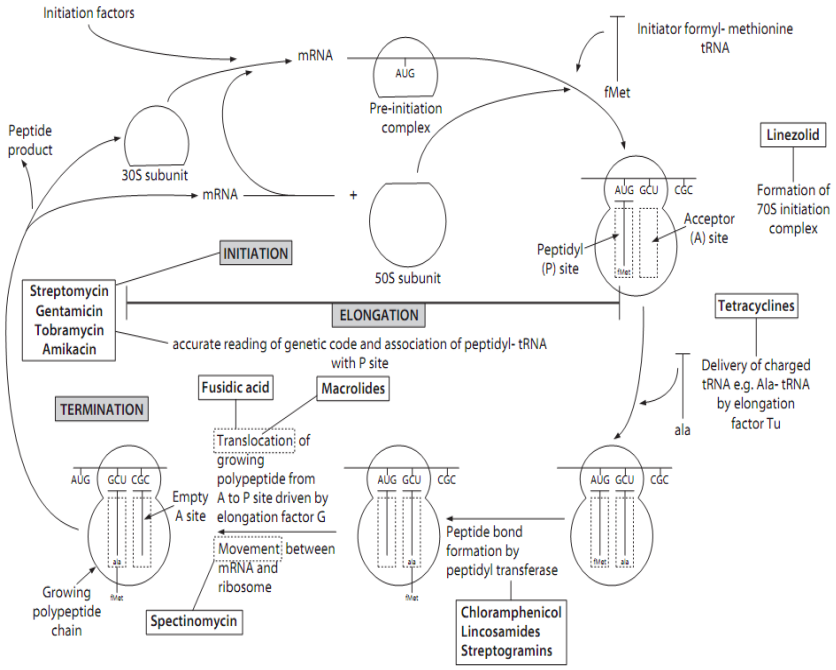

Figure 16: Protein biosynthesis inhibition (Adapted from Kohanski *et al*., 2010).

iii. Inhibition of nucleic acid synthesis

The replication process allows DNA reproduction, an essential step allowing microbial multiplication (Yoneyama and Katsumata, 1996; Džidič *et al*., 2008; Kohanski *et al*., 2010). Bacterial DNA is coiled in the cell and requires changes in

structure during the replication process. The topoisomerases are one of the enzymes that changes the shape of the DNA, a process required in DNA synthesis (Yoneyama and Katsumata, 1996; Džidič *et al*., 2008; Kohanski *et al*., 2010). The quilonone antibiotic targets topoisomerases II (DNA gyrase) and topoisomerase IV interferes with the maintenance of chromosomal topology. It binds these enzymes at the DNA cleavage stage and prevents DNA strand rejoining. While the quilonone-topoisomerase-DNA complex is formed, DNA replication becomes arrested at a blocked replication fork, leading to inhibition of DNA synthesis which leads to bacteriostasis and eventually to cell death (Kohanski *et al*., 2010). Various antibiotics such as Cip, levofloxacin and gemifloxacin are involved in DNA inhibition (Džidič *et al*., 2008; Kohanski *et al*., 2010). The sulfonamides (e.g. sulfamethoxazole) and trimethoprim block the key steps in folate synthesis, which is a cofactor in the biosynthesis of nucleotides involved in the synthesis of nucleic acids (Džidič *et al*., 2008).

The action of quinolones on bacterial DNA inhibition is presented in Figure 17.

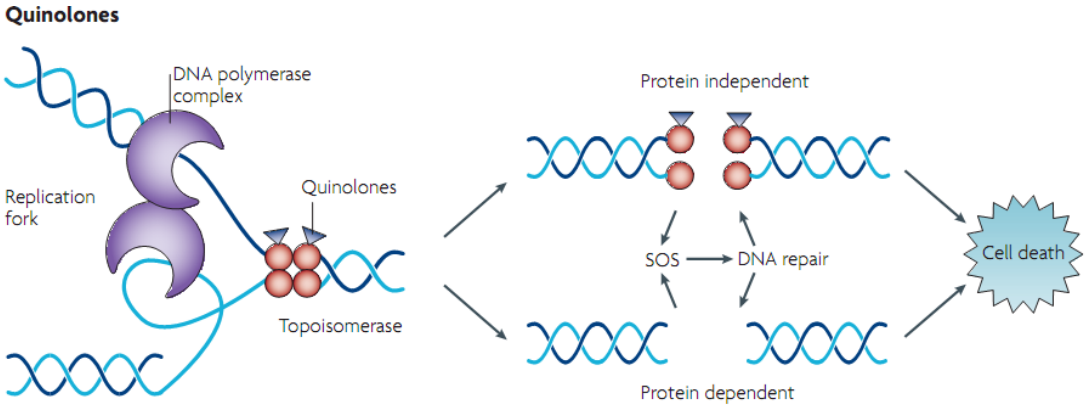

Figure 17: Action of quilonones on DNA inhibition (Adopted from Kohanski *et al*., 2010)

4.2.2.3. Mechanisms of antibiotic resistance in bacteria

i. Genetic mechanism of resistance

Environmental bacteria have evolved resistance mechanisms in order to avoid the inhibitory action of antibiotics (Mudryk, 2002 and Abdo *et al*., 2010). The development of antibiotic resistance is linked to the capacity of bacterial DNA to

mutate and/or to receive antibiotic resistance genes from other microorganisms (Abu and Egenonu, 2008; Blair, 2010). Resistance to antibiotics in bacteria may be natural or acquired and it is transmitted both horizontally and/or vertically in microbial communities (Spengler, 2006; Blair, 2010). Genetic mutations that occur and cause change in the bacterial DNA result in the newly acquired genes being able to be transferred among microbial community by several ways including conjugation, transformation and transduction (Tortora *et al*., 2010).

Conjugation is the most important and common mechanism of resistance transfer in bacteria. This mechanism is mediated by plasmid, a circular fragment of DNA, which replicates independently of bacterial chromosomes (Alanis, 2005; Spengler, 2006). The transmission of plasmids among bacteria is allowed via the formation of a pilus (hollow tubular structure) that forms between neighboring bacteria (Tortora *et al*., 2010). This temporary connection allows the passage from one bacterium to another of DNA encoding resistance genes (Blair, 2010).

Transformation is a form of DNA transfer involving direct passage of free DNA (naked DNA) from one bacterial cell to another (Alanis, 2005). The naked DNA usually originates from a dead and lysed bacterium. The receiving bacterium then introduces the free DNA into the cytoplasm and incorporates it into its own DNA (Tortora *et al*., 2010).

Transduction is a genetic transfer mechanism among bacteria and it occurs via the use of a ‘’vector’’, most often viruses capable of infecting bacteria. These viruses are also known as bacteriophages (Alanis, 2005 Spengler, 2006) so that when a bacteriophage destroys its current host it may take up resistance genes from the destroyed bacteria. When the virus, that now contains bacterial genes that encode antibiotic resistance, invades a new bacterium, it introduces this genetic material from the first bacterial cell into the receiving bacterium (Spengler, 2006 and Tortora *et al*., 2010).

ii. Biological mechanisms of antibiotic resistance

Biological resistance to antibiotics in bacteria is linked to the easy capacity of mutations in genes and the transfer of genetic material from one bacterium to another (Stuart and Marshall, 2004). The expression of such genes results in the

phenotype allowing resistance to antibiotics (Alanis, 2005; Tenover, 2006). There are four biological mechanisms of antibiotic resistance in bacteria that include: (i) the inactivation of antibiotics; (ii) the modification of target site, (iii) efflux of the antibiotic and, (iv) metabolic bypass (Blair, 2010).

Metabolic bypass is the mechanism through which bacteria become resistant to antibiotics whereby a reaction is circumvented by recruiting novel enzymes to bypass the metabolic pathway that is inhibited by antibiotic treatment (Blair, 2010). The best characterized example of resistance by metabolic bypass is the acquisition of PBP2a, an alternative PBP in methicillin-resistant *Staphylococcus aureus (S. aureus)*. This novel enzyme is encoded by the *mecA* gene. The PBP2a enzyme produced in addition to the other PBPs of *S. aureus* is not inhibited by antibiotics such as methicillin or other beta-lactams (Blair, 2010).

Antibiotic inactivation or modification: The defense mechanism in the category of antibiotic inactivation consists of the production of enzymes that modify antibiotic drug composition (Stuart and Marshall, 2004). Biochemical strategies used to achieve resistance include hydrolysis of the antibiotic, the transfer of chemical groups to antibiotics and a redox mechanism that changes the structure of the antibiotic so that they are then unable to bind target bacteria (Vỏ Thi Trά An, 2007; Blair, 2010).

Many antibiotics have hydrolytically susceptible chemical bonds such as esters and amide bonds. Bacterial enzymes target and cleave such bonds in the antibiotic molecule and, thus, reduce antibiotic activity (Stuart and Marshall, 2004). The classic hydrolytic amidase is β-lactamase that cleaves the β-lactam ring of penicillin and cephalosporin antibiotics before they can bind the target site in bacteria (Tonover, 2006; Blair, 2010).

Other resistant bacteria synthesize enzymes such as transferases which inactivate antibiotics such as the aminoglycosides, chloramphenicol, streptogramin, macrolide and rifampicin by chemical substitution. Thus, adenylyl, phosphoryl and acetyl groups are added to antibiotic molecules to modify them so that they become unable to bind to the target site in the bacterium (Stuart and Marshall, 2004). The oxidation or reduction of antibiotics is frequently exploited by pathogenic bacteria to inactivate the drugs. For example, tetracycline is oxidized

by the TetX enzymes secreted by bacteria. *Streptomyces virginiae* secretes antibiotics (A streptogramin and Virginiaycin M1) and protects itself from its own antibiotics by reducing ketone group of the drug to alcohol at the position 16, thereby making them inactive (Stuart and Marshall, 2004).

Target modification: Antibiotic resistance in bacteria is also achieved by modification of the targeted molecule to the antibiotic in order to render it insensitive to the antibiotic, while it still conserves its function in the organism (Blair, 2010). The modification of the antibiotic target molecule occurs either via spontaneous mutation or by acquisition of resistance genes (Blair, 2010) whose expression results in the alteration of antibiotic receptor sites that cannot allow antibiotics to bind tightly (Alanis, 2005). For example, quilonone resistance in *Salmonella spp* is caused by mutation in the *gyrA* gene that codes the A subunit of the DNA gyrase enzyme (Blair, 2010).

Antibiotic active efflux: The plasma membrane of bacteria holds efflux pumps (regulatory proteins) that export antibiotics outside the cell in order to keep their concentration at a low level (Alanis, 2005; Stuart and Marshall, 2004). Efflux pumps affect all classes of antibiotics, especially the macrolides, tetracycline and the fluoroquinolones because these antibiotics inhibit different aspects of protein and DNA biosynthesis (Spengler, 2006; Blair, 2010). Efflux pumps vary in their specificity and mechanism and many efflux systems confer multidrug resistance (MDR) and expel a wide spectrum of structurally unrelated drugs from the bacteria (Spengler, 2006; Blair, 2010). According to Blair (2010), the plethora of efflux pumps associated with MDR have been classified into five families: (i) the ABC superfamily, (ii) the major facilitor superfamily (MFS), (iii) the multidrug and toxic compound extrusion (MATE) family, (iv) the small multidrug resistance (SMR) family and (v) the resistance nodulation division (RND) superfamily. This classification is based on the number of components from which the pumps are composed, the energy source, the class of substrate they transport and the number of membrane spanning regions (Blair, 2010). Some of these mechanisms are illustrated in Figure 18.

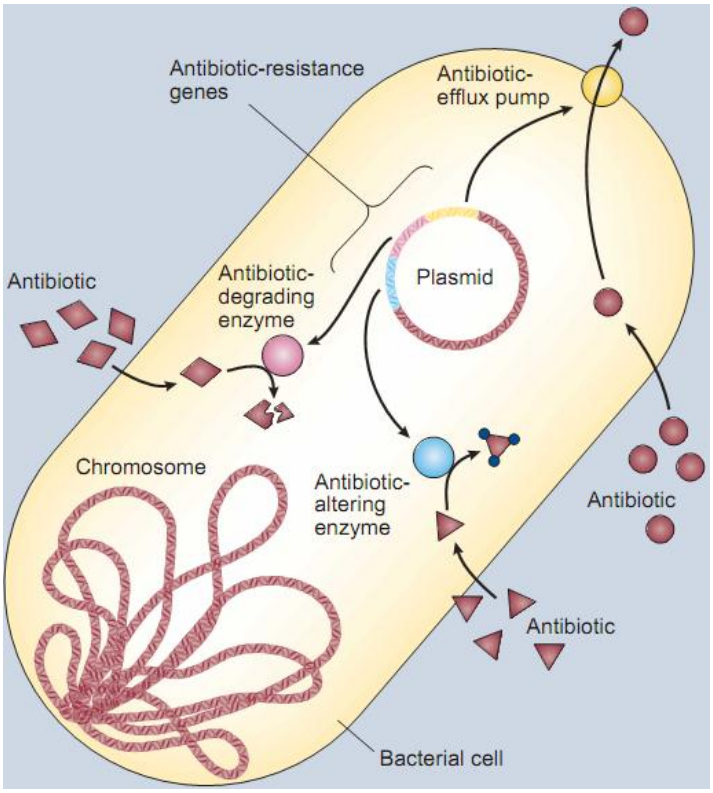

Figure 18: Antibiotic resistance mechanism in bacteria (Adopted from Stuart and Marshall, 2004)

4.3. Methodology

4.3.1. Study area and sampling process

The current research was conducted in the Kahwa River located in the Bukavu sub-basin. In order to achieve the research goals, samples consisting of sediment and water were collected from the sampling sites as described above.

While the current study focused on pathogenic bacteria, for completeness sake, attention was also paid to indicator bacteria. The isolated microbes were tested for their resistance to a selection of antibiotics as well as to HMs, particularly Cd and Pb. Bacteria resistant to both antibiotics and heavy metals were reported to play important environmental implications in the transfer of antibiotic resistance among microbial communities and the removal of the metals from polluted ecosystems (Spain and Alm, 2003)

4.3.2. Indicator bacteria

4.3.2.1. Sampling process

i. Water sampling

The samples of water intended for the indicator bacterial analysis were collected in duplicate as described by Hamid *et al*., (2007). Samples were collected away from the river edges in 500 ml sterilized bottles at each of the sampling sites. In the process, sterile plastic bottle was submerged in river water to a depth of 15 to 30 cm while pointing the mouth of the bottle toward the direction of the river flow. The bottle was filled up leaving an air space to allow for adequate sample mixing prior to analysis and the cap was replaced before the bottle was removed from the water. Bottles were then covered within sterile aluminum foil to avoid sample contamination and they were stored in an ice bag at around 4°C to 6°C. The bacteriological analyses were carried out in the laboratory of the Congolese Control Office (CCO) within four to six hours after collection.

ii. Sediment sampling

The sediment destined for indicator bacterial analysis was collected in duplicate at each sampling site using sterile plastic pipes of 1.3 cm diameter and two metres in length. In the process, the pipes were rinsed with ethyl alcohol and distilled water before being pressed in the river's bed. The sediment samples was transferred into sterile bottles covered within sterile aluminum foil to avoid sample contamination. Each was stored in an ice bag at low temperature (around 4°C) (Nkurikiyimfura *et al*., 2001). Bacteriological analysis was performed within six hours of collection in the laboratory of the CCO.

Precautions were taken to avoid confusion between samples and included labeling of samples with the site code, sampling date, the type of sample and the type of analysis designed for the sample.

4.3.2.2. Sample process and isolation of bacteria

i. Isolation of indicator bacteria in water

The bottles of sample water were shaken vigorously to mix the sample adequately. Then, 1 ml was used to prepare a dilution series from 10^{-6} to 10^{-8} as described by Nkurikiyimfura *et al*. (2001); Hamid *et al*., (2007) and Bahati *et al*. (2013).

The isolation of faecal coliforms was performed as follows: 1 ml of each diluted sample was filtered through three parallel filter membranes (0.45 µm pore size) as described by Ntabugi (2012) and Bahati *et al*., (2013). Thereafter, the membranes were placed onto tergitol 7 agar supplemented with tetrazolium chloride (TTC) (1%) (10:1) and then incubated at 44.5°C for 24 hrs. The resulting yellow, orange or brick-red colonies with a yellow central halo growing on the filter membranes were identified as being faecal coliform bacteria (Nkurikiyimfura *et al*., 2001).

The isolation of total coliforms was performed as described above. However, the plates were incubated at 37°C for 24 hrs. Yellow, orange or brick-red colonies with a yellow central halo, growing on the membranes, were identified as being total coliforms (Nkurikiyinfura *et al*., 2001).

The isolation of *Escherichia coli* was performed as described above. In the process, filter membranes were placed on the surface of MacConkey agar at 37°C for 24 hrs. Red colonies formed on the membrane were recorded as being *Escherichia coli* (Nkurikiyimfura *et al*., 2001; Bahati *et al*., 2013)

The isolation of *Enterococci*: This was performed as described above. Briefly, the filter membranes were placed on the surface of Slanetz and Bartly agar supplemented with TTC (1%) for 24 hr at 37°C (Lotter, 2010). The red-black colonies growing on the membrane were identified as being *Enterococci* (Nkurikiyimfura *et al*., 2001; Ntabugi, 2012; Bahati *et al*., 2013)

ii. Isolation of indicator bacteria in sediment.

The isolation of coliform in sediment was performed as follows: 10 g of sediment was added to 90 ml of buffered peptone water (BPW) and stirred thouroughly. Then, 1 ml of the mixture was used to prepare a dilution series from

10^{-6} to 10^{-8} as described by Nkurikiyinfura *et al*. (2001). Thereafter, 1 ml of each dilution was filtered through three parallel filter membranes. The isolation of the bacterial colonies then followed as described above

4.3.3. Pathogenic bacteria

4.3.3.1. Sampling and sample process

i. Water sampling

The sampling of pathogenic bacteria in water was performed either by using sterile swabs or by centrifugation methods. In the process, the swabs made of cheese cloth (23 cm wide, folded five times at 36 cm lengths, and cut lengthwise to within 10 cm from the tip into strips approximately 4.5 cm wide), were placed in duplicate for two days at each sampling site between 15 and 20 cm beneath the surface of water (Ntabugi, 2012). They were then removed and placed into separate sterile bottles, covered within aluminium foil and stored in ice bags at around 4^{o}C. The swabs were then transported to the laboratory of the CCO where they were analysed within six hours of collection.

The centrifugation method was used in the isolation of *Shigella* spp in water. Samples (500 ml) of water, collected in duplicate, were shaken and centrifuged separately at 1520 *g* for 15 minutes. The supernatant was filtered through sterile filter membranes (0.45 µm pore size) with the exception of the last 10 ml (Butler, 1968). Then, the membrane filter and the remaining 10 ml were mixed together for further analysis.

ii. Sediment sampling

The sediment designed for the isolation of pathogenic bacteria was sampled as described above**.**

4.3.3.2. Isolation of pathogenic bacteria

i. Isolation of pathogenic bacteria in water

Isolation of *Salmonella* spp in water required sterile swabs, taken up from water, being weighed and then mixed with sterile BPW diluted 1:10 (1:10). These were incubated in a water bath at 44°C for 5 mins to reactivate the stressed bacteria and then placed in an incubator at 37^{o}C overnight (Nkurikiyinfura *et al*.,

2001; Environment Protection Agency, 2004; David, 2010). Then, 5 ml aliquots of the enrichment broth were transferred to Rappaport Vassiliadis (RV) enrichment broth (1:10) and incubated at 37°C for 24 hrs (CDC, 2003; Environment Protection Agency, 2004; Thi Thu Hao Van, 2007, Lotter, 2010). After incubation, the broths were streaked in parallel onto five xylose lysine desoxycholate (XLD) agar and the plates were then incubated at 37°C for 24 hrs (Environment Protection agency, 2006; Lotter, 2010). The red-smooth colonies (2-3 mm in diameter) with or without a black centre or the wholly black colonies were identified as being *Salmonella* spp (Perilla *et al*., 2003; Environment Protection Agency, 2004;Thi Thu Hao Van, 2007, Lotter, 2010).

Thereafter, five well isolated suspected colonies of *Salmonella* spp were selected and streaked separately onto XLD agar. They were identified by both Kligler Iron Agar (KIA) and Triple Sugar Iron agar (TSI) biochemical tests and the gram stain. In the process, the centre of an isolated colony was lightly touched with an inoculum needle before being inoculated into KIA and TSI agar. The inoculation was performed by stabbing the butt of the medium followed by the streaking of the slant surface. The plates were incubated for 10 to 18 hrs at 37^{o}C and observed for typical *Salmonella* spp reaction (Perilla *et al*., 2003). *Salmonella* spp reactions were guided by Table 9. Three of the well isolated and identified colonies were stored as described by Cheryl *et al*., (2002) for further tests of antibiotic and HMs resistance.

Table 8: Biochemical reaction of *S. typhi* and *S. paratyphi* (Perilla *et al*., 2003)

Medium	***S. typhi* A**	***S. paratyphi***
Triple sugar agar (TSI)	K/A(+)	K/AG-
Kligler iron agar (KIA)	K/A(+)	K/AG-

Where: **K**: alkaline slant (red); **A**: acid butt (yellow); **G**: gas production; **+**: H_2S black (weak reaction); **-**: H_2S negative (no H_2S produced)

Isolation of *Shigella* spp was performed as follows: A total of 500 ml of water was collected in duplicate at each site. Each sample was centrifuged at 1520 *g* for 15 min before the supernatant was filtered through a sterile membrane

(0.45 µm pore size) except for the last 10 ml. Thereafter, the filter membrane and the last 10 ml were added together, weighed and mixed with selenite enrichment F broth (1:10) before being incubated at 37^{o}C for 21±3 hrs (Butler, 1968). The enrichment broths were streaked in parallel onto five XLD agar and the plates were incubated at 37^{o}C for 21±3 hrs. After the incubation period, small pink-red or colorless colonies were identified as being *Shigella dysenteriae* (Cheryl *et al*., 2002; Perilla *et al*., 2003; Environment Protection Agency, 2006).

Five well isolated suspected *S. dysenteriae* colonies were further characterized by KIA and TSI biochemical tests and gram stain as described above. Three well isolated and identified colonies were stored as described by Cheryl *et al*., (2002) to be tested for their resistance to antibiotics and HMs.

Biochemical reactions of a typical *S. dysenteriae* in KIA and TSI are presented in Table 10 (Perilla *et al*., 2003; Cheryl *et al*., 2002).

Table 9: Biochemical reaction of *S. dysenteriae* (Cheryl *et al*., 2002; Perilla *et al*., 2003)

Medium	**Characteristic reactions**
Triple iron agar (TSI)	K/A, no gas, no H_2S
Kligler iron agar (KIA)	K/A, no gas, no H_2S

Where,

K: alkaline slant (red); **A**: acid butt (yellow)

Isolation of *Vibrio* spp was performed as follows: Sterile swabs were removed from the river water, weighed and put into a bottle containing alkaline peptone water (APW) (1:1). They were incubated at 35^{o}C for 6 to 8 hrs (Cheryl *et al*., 2002; Perilla *et al*., 2003). After incubation, the enrichment broth was streaked in parallel onto five thiosulphate citrate bile salt sucrose (TCBS) agar and the plates were incubated at 37^{o}C for 18 hrs (Cheryl *et al*., 2002; Perilla *et al*, 2003; Tomatcho *et al*., 2009). Yellow colonies (2-4 mm in diameter) on the plates were considered as being *Vibrio cholerae* (Cheryl *et al*., 2002; Perilla *et al*., 2003). Thereafter, five well isolated colonies were selected and subcultured onto five separate plates. They were further identified by KIA and TSI tests. In the process,

the centre of isolated colony was touched and streaked onto the surface of brain heart infusion agar (BHIA) incubated at 35-37^{o}C for 24 hrs (Cheryl *et al*., 2002; Perilla *et al*., 2003). The colonies grew on HIA plate were touched gently using sterile inoculating needles and inoculated into KIA, and TSI as described above. Three of the five pure plates identified colonies were stored as described by Cheryl *et al*. (2002) and were tested for their resistance to antibiotics and HMs.

The biochemical reactions of *V. cholerae* are represented in Table 11 (Cheryl *et al*., 2002; Perilla *et al*., 2003).

Table 10: Biochemical characteristics of *V. cholerae* (Cheryl *et al*., 2002; Perilla *et al*., 2003)

Medium	**Characteristic reactions of V. cholerae**
Triple iron agar (TSI)	A/A, no gas, no H_2S
Kligler iron agar	K/A, no gas, no H_2S

Where; K: acid (red), A: alkaline (yellow)

ii. Isolation of pathogenic bacteria in sediment

Isolation of *Salmonella* spp in the sediment was performed according to methods described by Nkurikiyimfura *et al*. (2001) and Thi Thu Hao Van (2007). In the process, 25 g of sediment was added to 225 ml of BPW (pre-enrichment broth) and agitated thoroughly. The mixture was incubated first in a water bath for 5 min at 44°C to revive *Salmonella* spp. stressed by the external environment and then at 37°C overnight. An aliquot of 5 ml of the pre-enrichment broth was transferred into a tube containing 50 ml of RV and incubated at 42°C for 18 to 24 hrs. The process followed as described for the water samples.

Isolation of *Shigella* spp in sediment was performed as follows: 25 g of the sediment was mixed with 225 ml of BPW (1:10), and the mixture was shaken vigorously and incubated at 37^{o}C for 6 hrs. Thereafter, the broth was transferred to selenite F broth (1:1) and incubated at 37^{o}C overnight (Cheryl *et al*., 2002; Perilla *et al*., 2003). The processes were followed as described in the isolation of *Shigella* spp in water.

Isolation of *Vibrio* spp in sediment was performed as follows: 25 g of sediment was transferred to 225 ml of APW incubated at 37°C for 8 hr. After the incubation period, separate loopfulls of enrichment broth were streaked in parallel onto five TCBS agar incubated at 37°C for 24 hrs (Cheryl *et al*., 2002; Perilla *et al*., 2003). The suspected *V. cholerae*, characterized from their morphology (color and shape) were further identified by biochemical tests of KIA and TSI as described in the section above.

4.3.4. Antibiotic susceptibility test

Antibiotic susceptibility tests on bacteria isolated in sediment and water in the wet and dry seasons were performed by the disk diffusion method (DDM) on Mueller Hinton (MH) agar, prepared according to manufacturer's instructions. The standard procedure of the Clinical and Laboratory Standard Institute (CLSI) was strictly followed throughout the testing procedure. The prepared MH agar medium was cooled and poured into Petri dishes to a depth of 4 mm. They were allowed to solidify for 10 to 30 minutes in the oven at 35^{o}C (Thi Thu Hao Van, 2007). The antibiotic susceptibility test of *Vibrio* spp, especially, required the addition of sodium chloride (NaCl) to the MH to a final concentration of 1%. As described by Thi Thu Hao Van, (2007), this NaCl produced optimal growth and did not have any effect on the diffusion rate of the anti-microbial agents.

The inoculation of the medium was performed as follows: each of three isolated and identified pathogenic bacterial colonies of each species and three other colonies of each indicator bacteria were touched lightly with separate sterile toothpicks and transferred into separate, non-inhibitory sterile brain heart infusion broths (BHIB) and incubated in the test tubes at 35°C overnight (Perilla *et al*., 2003; Thi Thu Hao Van, 2007; Manisha *et al*., 2011). The density of the culture was adjusted to a turbidity equivalent of 0.5 McFarland by adding sterile BHIB. Thereafter, a sterile swab was dipped into the BHIB and pressed firmly against the inside wall of the tube above the fluid level to remove excess liquid (Cheryl *et al*., 2002; Perilla *et al*., 2003). The swab was spread over the entire surface of the MH agar three times, rotating the plate approximately 60° after each application to ensure an even distribution of the inoculum. At the end of the process, the swab

was moved around the edges of the agar to remove excessive inoculum (Perilla *et al*., 2003; Thi Thu Hao Van, 2007; Blair, 2010). The antibiotics that were used in this research study were chosen in order to include at least the majority of the mode of action of antibiotics and, secondly, as they were used frequently in Bukavu Town to treat infections related to these microbes. The antibiotics selected included: Ampicillin (10 µg), Chloramphenicol (30 µg), Cotrimoxazole (1.25/23.75 µg), Ciprofloxacin (5 µg) and Tetracycline (30 µg). These discs were stored at low temperature (4°C - 8°C) before being left unopened at room temperature for 1 hr before being used, in order to allow them to reach ambient temperature. Within 15 min after plating the bacteria, antibiotic discs were applied to the surface of the plates with sterile forceps and gently pressed down to ensure complete contact with the agar surface (Perilla *et al*., 2003; Thi Thu Hao Van, 2007; Manisha *et al*., 2011). To avoid overlapping zones of inhibition and a possible error in taking the measurements, the disks were distributed separately one from another by a distance no less than 24 mm from the centre and from the margin of the Petri dish wall (Perilla *et al*., 2003; Thi Thu Hao Van, 2007). Thereafter, the Petri dishes were inverted and incubated at 37^{o}C overnight. The control test consisted of disks cut from filter paper which had been dipped in distilled water and sterilized in the oven at a temperature of 150°C for 3 hrs.

Zones of inhibition were measured in millimeters with a ruler and recorded. From the diameter of the inhibition zones measured, bacteria were judged as being sensitive, of intermediate sensitivity or resistant. The inhibition zone size interpretive standards for bacteria of the family enterobacteriaceae for selected antimicrobial disks are presented in Table 12

Table 11: Inhibition zone size interpretive standards for enterobacteriaceae for selected antimicrobial disks: (Cheryl *et al*., 2002; Perilla *et al*., 2003).

	Diameter of inhibition zone (mm)		
Antimicrobial	**Susceptible**	**Intermediate**	**Resistant**
Ampicillin	≥17mm	14-16mm	≤13mm
Chloramphenicol	≥18mm	13-17mm	≤12mm
Cotrimoxazole	≥16mm	11-15mm	≤10mm
Ciprofloxacin	≥21mm	16-20mm	≤15mm
Tetracycline	≥19mm	15-18mm	≤14mm

4.3.5. Heavy metal tolerance

In order to assess the tolerance of bacteria to heavy metals, the disk diffusion and dilution methods were applied.

4.3.5.1. Disk diffusion method

The susceptibility test involving the interaction of HMs (Cd and Pb) with isolated bacteria was performed using the disk diffusion methods to assess tolerance towards Cd and Pb. Three well identified bacteria colonies of each species isolated were inoculated separately in the different test tubes containing BHIB and then incubated at 37°C overnight (Cheryl *et al*., 2002). The broth was spread onto MH agar and left for 15 min before sterile disks containing HMs were placed onto the plate (Mohd Ezhar *et al*., 2012). To prepare HMs disks, the filter paper was punched from the filter paper and each disk obtained (7 mm in diameter) was soaked for one hour to allow adequate absorption of the HMs into the disks in different solutions of HMs salts ($CdCl_2$ or $PbCl_2$) prepared at concentrations of 0.25, 0.5, 0.75, 1, 1.25, and 1.5 mg/ml. The disks were allowed to dry and then sterilized in the oven at 150°C for 3 hrs (Mohd Ezhar *et al*., 2012). Thereafter, the disks were applied onto the surface of each HM plate and incubated at 37°C for 48 hrs (Mohd Ezhar *et al*., 2012). To avoid overlapping zones of inhibition and a possible error in taking the measurements, the disks were distributed on the agar surface as described above.

The inhibition zone diameters were measured in millimeters with a ruler. For an inhibition zone size greater than 1 mm around the HM disk, the bacterium was recorded as being sensitive (Mohd Ezhar *et al*., 2012). The control test was made of filter paper soaked in distilled water for one hour, before being dried and sterilized in the oven at 150°C for 3 hrs before being applied onto the surface of the HM plate.

4.3.5.2. The growth methods

This was performed by growing the colonies of bacteria in duplicate on agar supplemented with HMs. In the process, metal salts ($CdCl_2$ or $PbCl_2$) were dissolved in distilled water to prepare metal concentrations from 0.25, 0.5, 0.75, 1, 1.25, to 1.5 $mg.ml^{-1}$ (Srikumaran *et al*., 2011; Mutuku *et al*., 2014) and bacteria were examined for their ability to grow on the media (Atuanya and Oseghe, 2006, Mutuku *et al*., 2014). The control tests were made of medium without metal but with bacterial inocula (bacterial growth control or biotic control) and medium with either Cd or Pb but without bacteria (abiotic control).

4.3.7. Statistical data analysis

The data obtained in the research were analyzed by xlstat software. Parametric test (t-test) and the Parkson correlation test were applied in the analysis of the results. The t-test allowed comparison between sampling sites in the concentration of bacteria. The difference was significant if a p-value was less than 0.05 ($p<0.05$). However, Pearson test applied to data allowed knowing correlation between Cd and Pb resistance in bacteria. The correlation was not significant when $p>0.05$.

4.4. Results and discussion

4.4.1. Results

4.4.1.1. Indicator bacteria

The sediment and water collected in the Kahwa River in the wet and dry seasons were diluted to 10^{-6}, 10^{-7} and 10^{-8} to allow the counting of the colonies growing on the plates as described above. The result obtained and presented in the sections below is the average of dilutions.

i. Isolation of indicator bacteria in water

Bacteriological analysis of water revealed that it was contaminated with *E.coli*, *Enterococci*, faecal and total coliforms (fig. 19), indicating faecal pollution by humans and animals (Melita *et al*., 2001; Lotter, 2010). *Enteroccocci* appeared to be the most abundant bacteria in samples collected from all the sampling sites. This might be explained by the lengthy survival of these bacteria outside the host (Lotter, 2010; Figueras and Borrego, 2010). A comparison between sampling sites as to the concentration of indicator bacteria in water did not reveal a significant difference ($p>0.05$). This might be explained by the sewage being simply discharged into the river and its tributaries (Fig. 6 and 9).

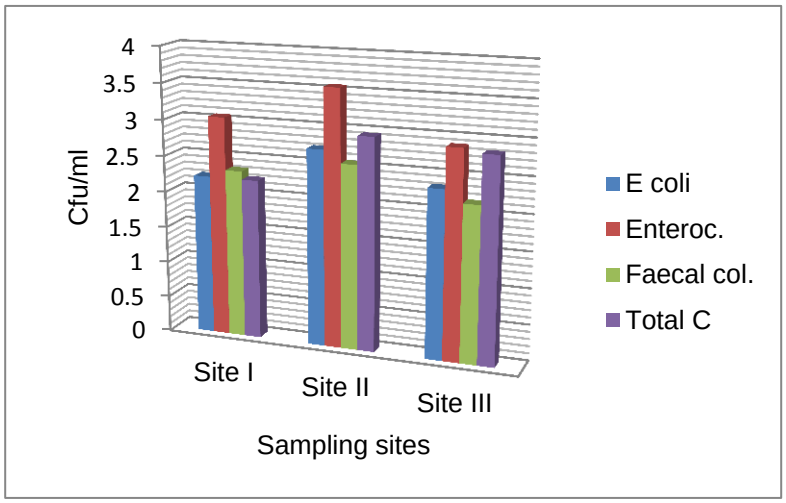

Figure 19: The average number of indicator bacteria in water

ii. Isolation of indicator bacteria in the sediment

The research carried out on the sediment to assess bacteriological contamination revealed that, as with the water, it was polluted by *E.coli*,

Enteroccocci, total and faecal coliforms (fig. 20), indicating human and animal faecal pollution (Melita *et al*., 2003; Bahati *et al*., 2013). The *Enteroccocc*i and total coliforms were found in high concentration at all the sites with the former bacteria being particularly prevalent at site II. Comparison between sampling sites as to the concentration of indicator bacteria in the sediment didn't show a significant difference ($p>0.05$) with the exception of *E coli* and faecal coliform between site I and site II and total coliform between site I and the relatively high concentration of these bacteria at site II and site III. This may be explained by the fact that the collected samples at all the sampling sites were contaminated with raw faeces fom both humans and animals and from the activities sited along the river and its tributaries.

However, a comparison between the sites as to the concentration of indicator bacteria in the sediment and water showed a significant difference ($p<0.05$) with exception to *E coli* in sites I and II and to faecal coliform in site I. This might be explained by the fact that the sediment contains high concentration of nutrients that may allow the survival and the multiplication of the microbes (Abdo *et al*. (2010). In addition, microbes dispersed in water may be affected by the sun's rays. Lotter (2010) reported that the sun's rays penetrating the surface water may affect the concentration of microbes.

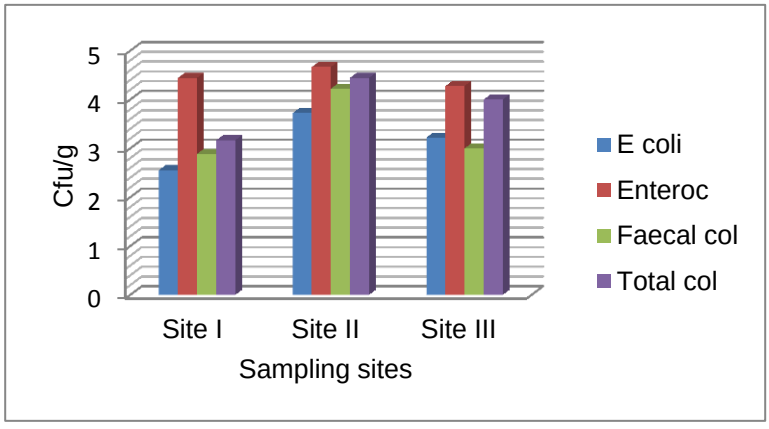

Figure 20: The average number of indicator bacteria in the sediment

4.4.1.2. Pathogenic bacteria

i. Isolation of pathogenic bacteria in water

The study related to pathogenic bacteria in water showed that it was contaminated by *Vibrio* spp., *Shigella* spp and *Salmonella* spp (fig. 21 and 22) and thus, it poses a health risk to humans and the biodiversity to which it is exposed (Hamid *et al*., 2007; Abdo *et al*., 2010). It was observed throughout the study that *Vibrio* spp. was the most abundantly isolated bacteria in all the sampling sites and this might be explained by the fact that water constitutes a natural habitat of *Vibrio* spp (Tamatcho *et al*., 2009). A comparison between the sampling sites as to the concentration of pathogenic bacteria did not show a significant difference ($p>0.05$) with the exception of *Shigella* spp and *Vibrio* spp at sites I and II.

The average number of pathogenic bacteria positive plates in water is presented in figure 21

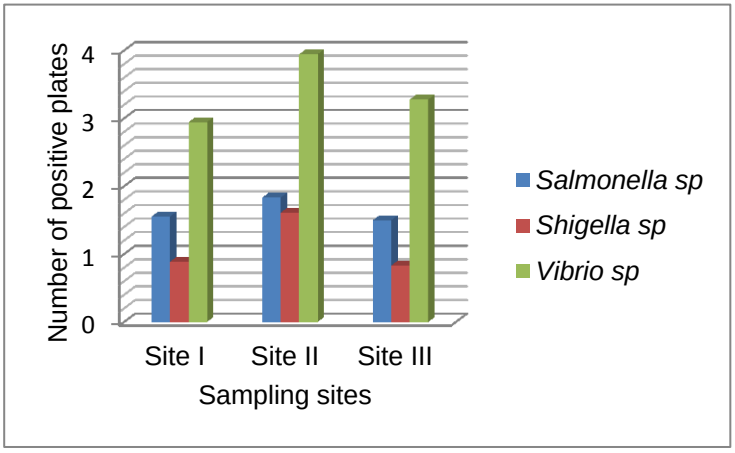

Figure 21: Average number of pathogenic bacteria in water

ii. Pathogenic bacteria isolated in the sediment

Bacteriological analysis of sediment collected in the sampling sites on the Kahwa River showed that it was also highly polluted by *Vibrio* spp, *Shigella* spp and *Salmonella* spp. *Vibrio* spp were most commonly isolated at all the sampling sites (fig. 22). A comparison between sampling sites as to the concentration of pathogenic bacteria did not reveal a significant difference ($p>0.05$) with the exception of *Vibrio* spp at sites I and II and I and III. This may be explained by the sewage being dumped into the river and its tributaries. Also, the comparison between water and sediment in the concentration of bacteria in the sampling sites

did not show a significant difference (p>0.05) with the exception of a relatively low level of *Salmonella* spp at site I.

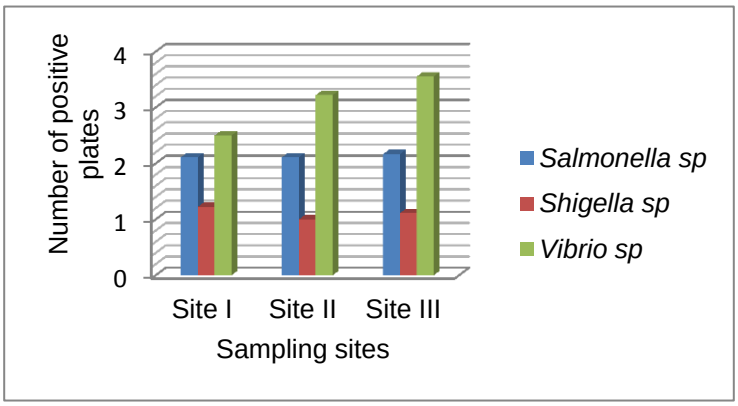

Figure 22: Average number of pathogenic bacteria in sediment

4.4.1.3. Identification of pathogenic bacteria by the KIA and TSI tests

i. identification of pathogenic bacteria isolated in water

Pathogenic bacteria isolated in water were identified using the Gram stain and colony morphological characteristics that were confirmed by biochemical tests (KIA and TSI) as described by Cheryl *et al*. (2002) and Perilla *et al*., (2003). The results obtained in the study showed that water was polluted by *S. typhi*, *S. paratyphi*, *S. dysenteriae* and *V. cholerae* (fig. 23), revealing that the river presents a danger to the health of the citizens of Bukavu Town as well as the animals living near to the river. A comparison between sampling sites as to the concentration of pathogenic bacteria did not show a significant difference (p>0.05).

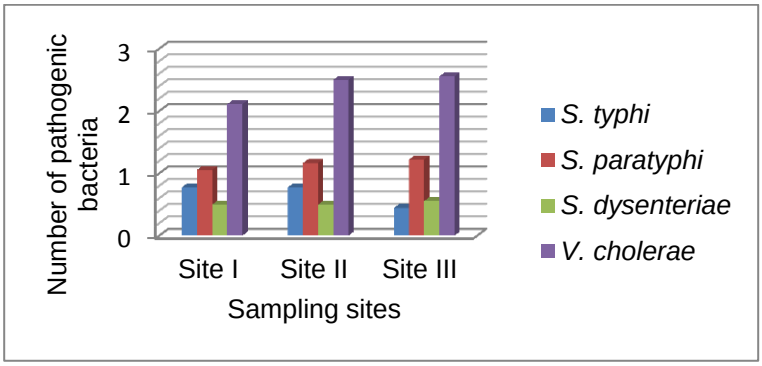

Figure 23: Average number of pathogenic bacteria in water

ii. Identification of pathogenic bacteria isolated in the sediment

The KIA and TSI biochemical tests used to confirm the presense of pathogenic bacterial colonies isolated in the sediment showed that it was polluted by *S. typhi*, *S. paratyphi*, *S. dysenteriae* and *V. cholerae*. As in the water samples, *V. cholerae* was the most commonly isolated bacterium (fig. 24). A comparison between sampling sites as to the concentration of pathogenic bacteria in the sediment did not reveal a significant difference ($p>0.05$) with the exception of *V. cholerae* at sites I and II noted at relatively low concentration compared to the *V. cholera*e noted at site III. Also, no significant difference was found when the sediment and water were compared as to the concentration of pathogenic bacteria. The average number of pathogenic bacteria in sediment is presented in figure 24.

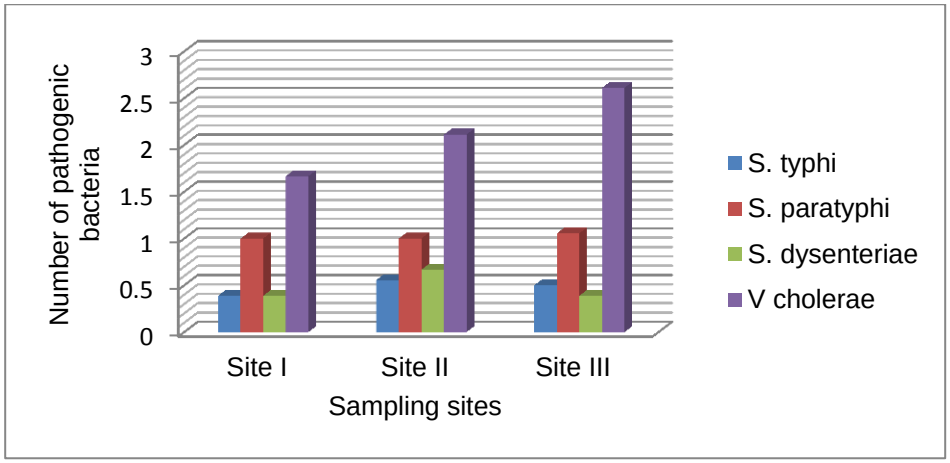

Figure 24: The average number of pathogenic bacteria in sediment

4.4.1.4. Antibiotic susceptibility test on bacteria isolated in the Kahwa River

i. Antibiotic susceptibility test on bacteria isolated water

The antibiotic susceptibility tests performed on bacteria isolated in water revealed that most of the bacteria showed a degree of resistance to the antibiotics tested (fig. 25). At all three sites, all the bacteria screened were shown to be resistant to ampicillin (Amp) and most bacteria, with the exception of a faecal coliform strain, were also resistant to cotrimoxazole (Sxt). Significant resistance was shown to tetracycline (Tet) with the exception of some strains of *Enterococc*i, faecal coliforms and *S. paratyphi*. A similar profile was shown against

chloramphenicol (C). On the other hand, most bacteria were sensitive to ciprofloxacin (Cip), with the exception of *S. paratyphi, V. cholerae* and *S. typhi* with some resistance being shown by *E coli* and by faecal coliforms.

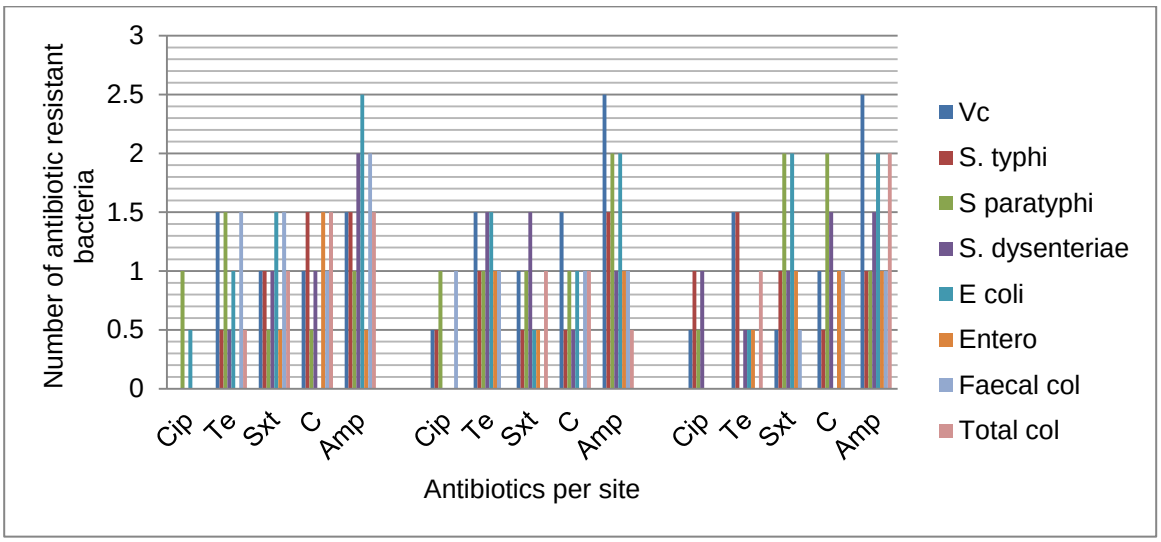

Figure 25: Relative growth in the presence of antibiotics of bacteria from water

ii. Antibiotic susceptibility test on bacteria isolated in the sediment

Research that was designed to assess antibiotic susceptibility on the part of bacteria isolated in the sediment showed that, compared to the bacteria isolated from the river water, relatively more sediment bacteria were resistant to the antibiotics tested. Statistically, a t-test applied to the data collected in sediment and water did not reveal a significant difference ($p>0.05$) with the exception of Te at sites I and II and Sxt and C at site II. Relatively more antibiotic resistance was demonstrated amongst bacterial strains such as *V. cholerae* and some of the *Salmonella* spp resident at site I as compared to the other two sites (fig. 26). As can be noted in figure 26, significant resistance was shown against Amp and to a slightly lesser extent to C, Sxt and Tet. More resistance to Cip was shown in the bacteria resident in the sediment compared to bacteria isolated from the water and an increase in resistance within and between bacterial strains was noted when comparing resistance in bacteria isolated from site I, then site II and finally site III. Thus, a comparison between sites as to the concentration of ARB revealed a significant difference ($p<0.05$) with the exception of Cip and C, respectively, at

sites I and III and I and II. Of these bacteria, particularly *V. cholerae* and the *Salmonella* spp showed resistance to ciprofloxacin while *E coli,* the *Enterococci* and coliforms showed reduced resistance to this antobiotic.

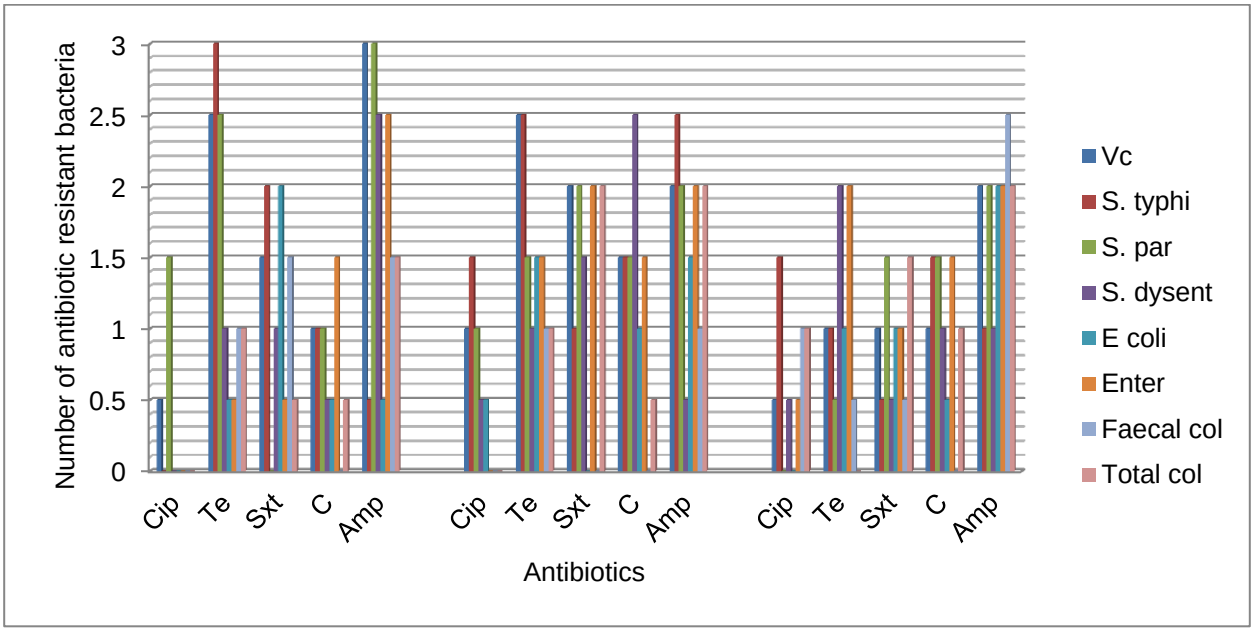

Figure 26: Relative growth in the presence of antibiotics of bacteria from sediment

4.4.1.5. Heavy metal susceptibility testing

i. Cadmium susceptibility test on bacteria isolated from water

The Cd susceptibility test performed on the bacteria isolated in water showed that most of the bacterial strains that were screened showed some tolerance to Cd (fig. 27). In general, it was observed that at a concentration between 0.25 mg/ml and 0.5 mg/ml, the number of Cd-tolerant bacteria was high and that this tolerance rapidly decreased at higher Cd concentrations. A comparison between sampling sites as to the concentration of Cd-tolerant bacteria screened with Cd at a concentration of 0.25 mg/ml did not reveal a significant difference ($p>0.05$) but a difference was noted when comparing site I bacterial tolerance after screening with Cd at a concentration of 0.5 mg/ml with those at sites II and III. Most of the bacteria collected at site I and treated with Cd at a concentration of 0.25mg/ml showed tolerance, with the exceptions being *S. dysenteriae, E. coli* and faecal coliforms. With the exception of *Enterococci*,

bacterial tolerance to Cd was lost at a Cd concentration of 0.5 mg/ml. At 1.5 mg/ml, tolerance was only observed in total coliforms. Relatively more tolerance to Cd was noted at site II and included most of the bacterial strains showing tolerance at both Cd concentrations of 0.25 mg/ml and at 0.5 mg/ml. The exceptions to this were sensitivity shown by *S. dysenteriae* at a Cd concentration of 0.25 mg/ml while at a Cd concentration of 0.5 mg/ml, only the faecal coliforms showed complete sensitivity to the Cd. At a Cd concentration of 1 mg/ml, tolerance was observed only in *S. typhi* and *Enterococci*. At site III, at a Cd concentration of 0.25 mg/ml, most of the bacterial strains showed tolerance with the exception of *S. paratyphi* and the faecal coliforms while at a Cd concentration of 0.5 mg/ml, tolerance was reduced and growth was not observed in *S. dysenteriae* and the *Enteroccocci*.

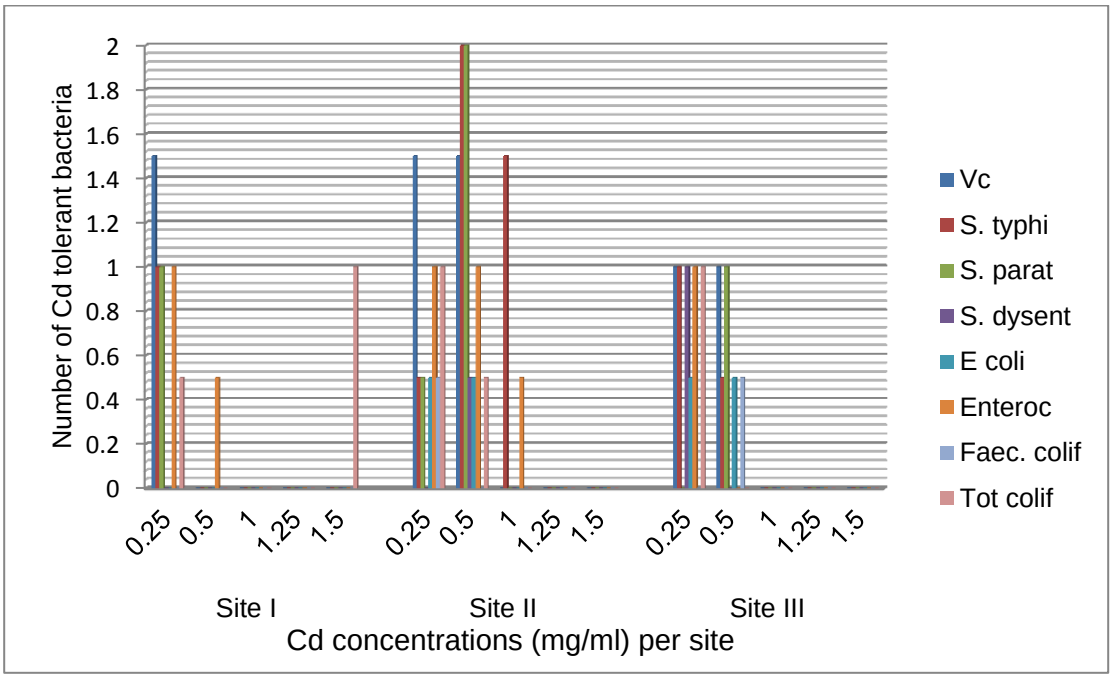

Figure 27: Average number of Cd-tolerant bacteria isolated in water

ii. Cadmium susceptibility tests on bacteria isolated in sediment

Figure 28 indicates the average number of Cd-tolerant bacteria in the sediment to show that most bacterial strains were tolerant to Cd. Significantly, this bacterial tolerance was evident at higher Cd concentrations compared to bacteria isolated from water collected at the same sites in the Kahwa River. At site I, all the bacterial strains showed tolerance to Cd up to a concentration of 1 mg/ml. At a Cd

concentration of 1.25 mg/ml, *E. coli* and faecal coliform were sensitive while at a Cd concentration of 1.5 mg/ml, only *V. cholerae, S. paratyphi* and *Enterococci* showed a low level of tolerance. At site II, tolerance showed a similar trend with a drop in tolerance shown at a Cd concentration of 1.25 mg/ml where sensitivity was shown in *S. dysenteriae* and faecal coliform. At 1.5 mg/ml, only *V. cholerae, S. paratyphi* and *Enterococci* showed tolerance. At site III, an exception to this trend was noted at a Cd concentration of 1.25 mg/ml where *S. typhi*, *E. coli* and faecal coliforms no longer showed tolerance and at a Cd concentration of 1.5 mg/ml only *S. dysenteriae* was still tolerant to the metal.

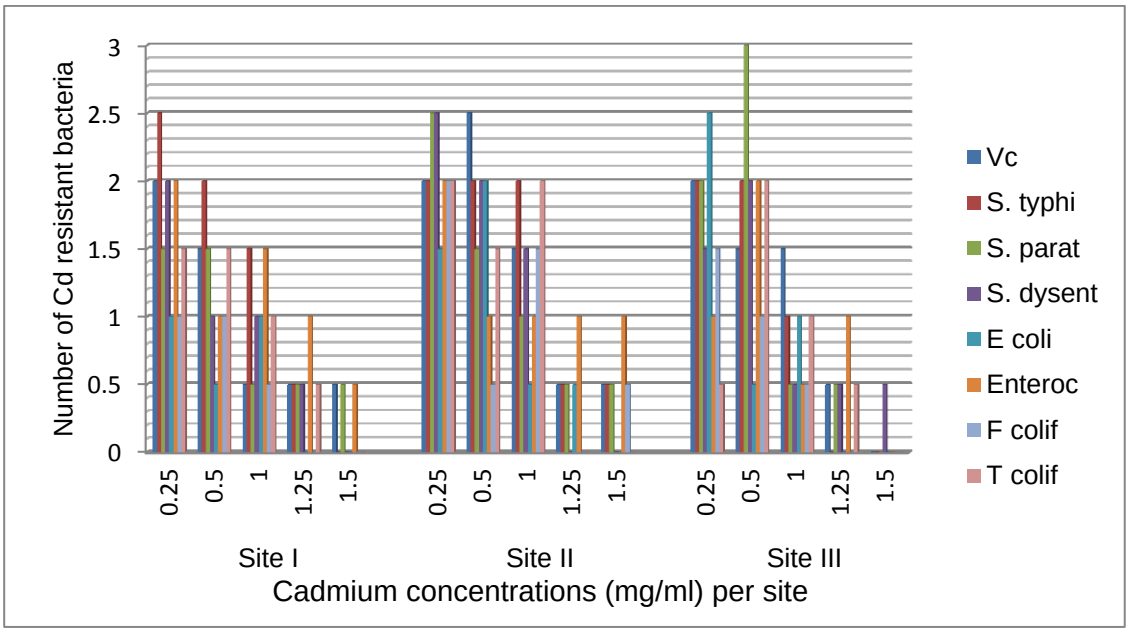

Figure 28: Average number of Cd-tolerant bacteria in the sediment

i. Lead susceptibility test on bacteria isolated in water

As indicated in figure 29, the study showed extensive tolerance to Pb on the part of bacteria isolated from water samples collected from the Kahwa River.

At site I, screening bacterial tolerance to increasing concentrations of Pb showed a sequential reduction in bacterial tolerance so that at a Pb concentration of 1 mg/ml, sensitivity to Pb was shown only by *E coli*. At higher Pb concentrations (1.25 mg/ml and 1.5 mg/ml) tolerance to Pb was restricted to *V. cholerae* and to *S. typhi and S. paratyphi*. Interestingly, in addition to these three tolerant bacterial strains, *Enterococci* showed tolerance to Pb at a Pb concentration of 1.5 mg/ml (but not at Pb concentrations at 1 mg/ml and 1.25 mg/ml). As shown in figure 27,

this strain of *Enterococcus* also showed a similar tolerance to Cd at a raised Cd concentration. At site II, all the bacterial strains showed tolerance to Pb to a concentration of 1.25 mg/ml. However, at a Pb concentration of 1.5 mg/ml, *E coli*, *Enterococci* and faecal and total coliforms lost tolerance. A comparison between the sampling sites as to the tolerance shown to various concentrations of Pb by the bacteria showed a significant difference ($p<0.05$) regarding the relatively low levels of bacterial tolerance shown at site I shown to Pb at 1 mg/ml and 1.25 mg/ml compared to sites II and III.

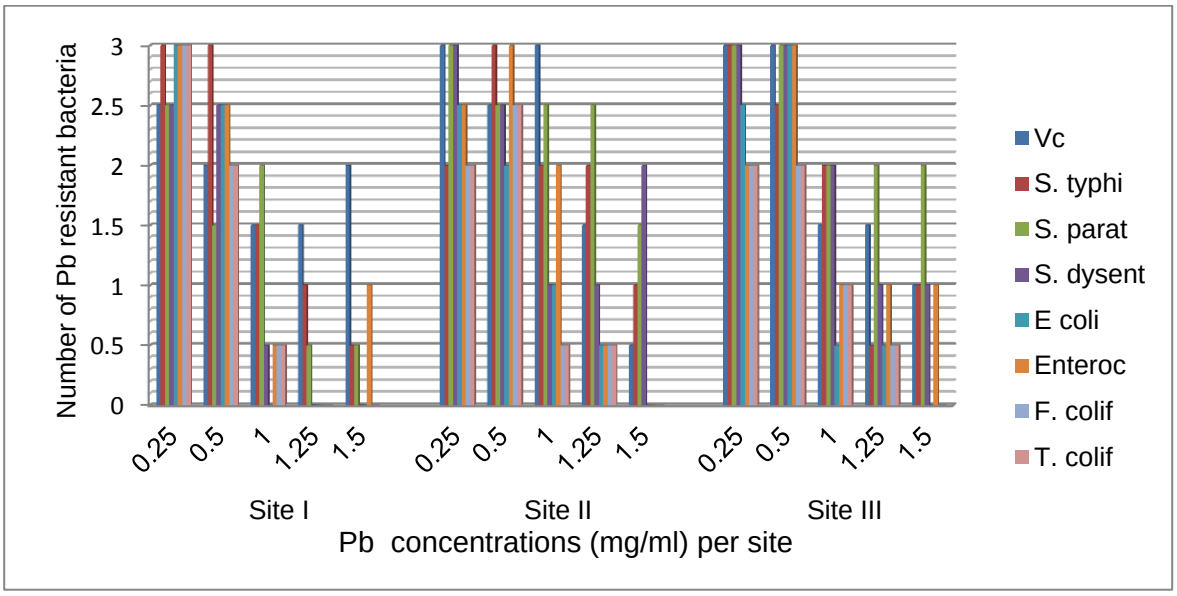

Figure 29: Average number of Pb-tolerant bacteria in water

ii. Lead susceptibility test on bacteria isolated in the sediment

Compared to Pb tolerance shown by bacteria isolated from water, the bacteria isolated from sediment showed that most were more tolerant to Pb (Fig. 30). This might be explained by the elevated accumulation rate of HMs in the sediment (Samir and Ibrahim, 2008; Akan *et al*., 2010; Nasrazadani *et al*., 2011). The relatively high levels of tolerance to Pb in bacteria isolated from sediment are shown in figure 30. At site I, *E. coli* was sensitive at a Pb concentration of 1.25 mg/ml. At a Pb concentration of 1.5 mg/ml, *E coli*, as well as *S. dysenteriae* and the coliforms were sensitive to Pb. At site II, all the bacteria showed tolerance to Pb, with the exception of *E coli* at a Pb concentration of 1.5 mg/ml. At site III, only *E coli* lost tolerance at a Pb concentration of 1.25 mg/ml. As expected, *E coli* were

sensitive to Pb at a concentration of 1.5 mg/ml and total coliforms lost tolerance to Pb at this concentration. A comparison between the sediment collection sites as to Pb-tolerant bacteria did not reveal a significant difference ($p>0.05$) except when comparing the tolerance shown to Pb at a concentration of 1.5 mg/ml at site I compared to the other two sites.

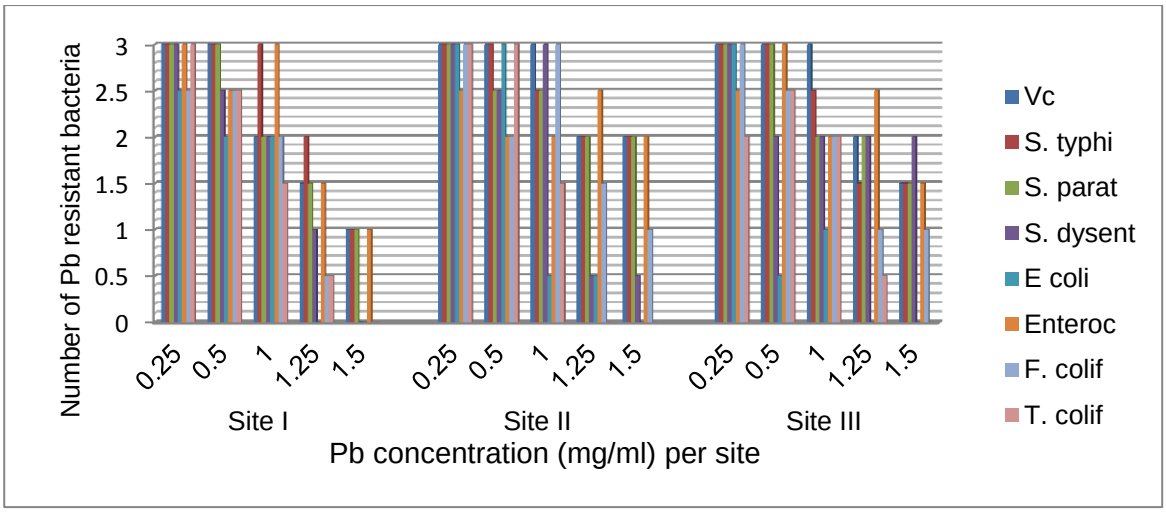

Figure 30: Average number of Pb-tolerant bacteria in the sediment

4.4.2. Discussion

4.4.2.1. Bacteriological pollution of the Kahwa River

The research carried out to assess bacteriological pollution in the Kahwa River revealed that its sediment and water were polluted by *E. coli*, *Enterococci*, faecal and total coliforms (fig. 19 and fig. 20) and the pathogenic bacteria (fig. 21 and fig. 22), particularly *V. cholerae*, *S. dysenteriae*, *S. typhi* and *S.* paratyphi (fig. 23 and fig. 24). These results indicate faecal pollution by both humans and animals (Carlender, 2006). Similar results were reported by Abdo *et al*. (2010) in the Ismalia canal in Egypt and by Lotter (2010) in the Western Cape in South Africa. The research carried out in the river catchment revealed that domestic and municipal effluent is thrown into the canals, the rivers and on the ground. Toilets, consisting in majority of pit latrines, are connected to canals and the Kahwa River and its tributaries. Thus, the bacteriological pollution of the Kahwa River may be explained by sewage from anthropogenic activities and households being discharged into the Kahwa River and its catchment. A number of authors have reported that municipal sewage and domestic effluent contains enteric bacteria

(Mudryk, 2001; Abdo *et al.* 2010; Czekalski *et al*. (2012). The discharge of such waste into aquatic ecosystems like the Kahwa River should lead to bacteriological pollution of the sediment and water of the river.

The *V. cholerae* and *Enteroccocci* were routinely isolated in the sediment and water collected at all the sites. This may be explained by the long survival of *V. cholerae* in rivers (Matatcho *et al*., 2009) and the reports indicating that *Enteroccocci* may live in aquatic environment for extended periods of time (Melita *et al*. 2003; Carlender 2006). The presence of such pathogenic bacteria in the Kahwa River suggests that they represent serious health risks.

4.4.2.2. Resistance of bacteria to selected antibiotics

Studies relating to antibiotic sensitivity are mostly performed in healthcare facilities rather than on environmental bacteria (Mudryk, 2002). The research carried out to assess antibiotic resistance on bacteria isolated in the Kahwa River showed that most isolated bacteria were resistant to selected antibiotics (fig. 25 and fig. 26). Untreated sewage input through effluent into the river might have contributed to the increase of antibiotic resistant bacteria in the sediment and water of the Kahwa River as reported by Thavasi *et al*. (2007) and Abdo *et al*. (2010). Czekalski *et al*. (2012) suggested that municipal waste contains antimicrobials such as heavy metals (HMs) and antibiotics. Licious *et al*. (2013), in research carried out on tannery effluent, reported that wastewater from human activities contain substances such as HMs and biocides that have the potential to select for antibiotic resistance even though they are not themselves antibiotics.

Bacterial antibiotic resistance reported in the Kahwa River may also be explained by exposure to harsh substances like HMs. The research carried out in the Kahwa River revealed that its sediment and water was polluted by both Cd and Pb (fig. 13 and fig. 14). According to Nasrazadani *et al*. (2011) and Lucious *et al*. (2013), bacteria evolving in an HMs-polluted environment develop resistance mechanisms that allow them to become resistant to compounds such as antibiotics. This may be explained by genes encoding resistance to both HMs and antibiotics being located close together on the same plasmid and may be transmitted together to nearby environmental bacteria (Nasrazadani *et al*., 2011;

Lucious *et al*., 2013). Mudryk (2002) and Czekalski *et al*. (2012) reported that the effluent from healthcare facilities is the major source of antibiotics into aquatic ecosystems and bacteria exposed to these antibiotics can develop resistance mechanism in an attempt to withstand environmental stresses. Yah Suh and Eghafona (2007) suggested that the misuse of drugs may lead microflora bacteria to develop drug resistance. Although they are harmless, they can become reservoirs for resistance genes for current and emerging pathogenic bacteria (Yah and Eghafona, 2007). As indicated above, there are various means whereby the genetic material in bacteria may change. Thus, adaptation shown by isolated bacteria may arise from mutation and/or the transfer of genes among microbial biofilm via mobile factors such as integrons, transposons or plasmids (Džidič *et al*., 2008; Manisha *et al*., 2011). Alternatively, it may occur through the acquisition of new genetic material from resistant microbes via conjugation, transduction and transformation (Džidič *et al*., 2008, Cezakalski *et al*., 2012).

The result of the current research showed a reduced bacterial resistance to Cip compared to other antibiotics in isolated bacteria (fig. 25 and fig. 26). These results correlate with the findings of Yah and Eghafona (2008). The low level resistance to Cip compared to other antibiotics may result from the fact that Cip is a relatively new antibiotic drug that is more expensive than other antibiotics such as tetracycline, ampicillin and chloramphenicol. Because of this, Cip might have a lower exposure to the environment, and, thus, a reduced chance of contact with microbes, and thus, a reduced chance of promoting the development of bacterial resistance to itself (Yah and Eghafona, 2008).

The resistance to antibiotics observed in bacteria isolated in the sediment and water of the Kahwa River may pose serious health problems to the town population and the biodiversity. Concentrated efforts between scientists, citizens and urban authorities are required in order to reduce the pollution of the Kakwa River and the subsequent transfer of resistance genes to other microbes.

4.4.2.3. Tolerance of bacteria to selected heavy metals

The amount of HMs, particularly Cd and Pb, has increased in bacterial aquatic ecosystems. This is largely due to industrial development, chemicalized

agriculture and population growth (Mutuku *et al*., 2014). Bacteria living in such a polluted environment can accumulate these metals (Nasrazadani *et al*., 2011). Once in the cytoplasm, Cd and Pb may disturb bacterial metabolic functions (Hynnien, 2010). In addition, they can damage bacterial cell membranes, enzymatic-specific properties and the structure of DNA (Nageswaran *et al*., 2012). Thus, bacteria are obliged to develop mechanism to tolerate the presence of these metals.

The research carried out in the Kahwa River to assess HMs tolerance in bacteria isolated in sediment and water showed that most bacterial isolates were resistant to Cd and Pb (fig. 27 to fig. 30). The tolerance to these HMs may be explained by the discharge into the river and its catchment of sewage and municipal waste. Mutuku *et al*., (2014) reported that domestic effluent and municipal waste discharge contains both bacteria and HMs. This was also reported by Barifaijo *et al*. (2009) and Nasrazadani *et al*. (2011). Thus, the discharge of waste into the Kahwa River and its catchment can lead to serious pollution of the sediment and water of the River. The tolerance to HMs shown by the microbes isolated in the Kahwa River may be explained by the fact that these microbes were already living in sediment and water that is heavily polluted with HMs. Similar results were reported by Nasrazadani *et al*. (2011) and Nageswaran *et al*. (2012). The adaptative characteristics developed by bacteria evolving in an HMs-polluted environment may be acquired through mutations and/or via the transfer from HMs-tolerant microbes of resistance genes by the transfer of plasmids to adjacent bacteria..

It was observed throughout this research that the number of bacteria growing in the presence of HMs decreased along with an increase in the concentration of the metal, revealing the toxic character of HMs at high concentrations. This was reported by Atuanya and Oseghe (2006) and Mutuku *et al*. (2013). In most cases, a correlation as to the tolerance of bacteria to Cd and Pb was not significant ($p>0.05$), indicating that Cd and Pb have different toxicity levels in the presence of microbes.

4.5. Conclusion

The current research aimed to assess bacteriological pollution in the Kahwa River. In order to achieve the research objective, indicator and pathogenic bacteria were isolated in the sediment and water samples that were collected using standard methods. The isolated bacteria were identified from their morphology, while pathogenic bacteria were further identified by biochemical tests using KIA and TSI as described by Cheryl *et al*. (2002) and Perilla *et al*. (2003). The HMs-tolerance tests was performed on the isolated bacteria as described by Atuanya and Oseghe (2006); Mohd Ezahar *et al*. (2012); Nageswaran *et al*. (2012) and Mutuku *et al*. (2013), while antibiotic susceptibility tests were described by Mudryk, (2002) and Manisha *et al*. (2011).

The results obtained in the current investigation revealed that the Kahwa River was extensively polluted by indicator bacteria (*E. coli*, *Enteroccocci*, faecal and total coliforms) as well as pathogenic bacteria such as *V. cholerae*, *S. dysenteriae*, *S. typhi* and *S. paratyphi*l. These findings are of concern not only because of the misuse of the Kahwa River by people living along its banks and the need to train the citizens of Bukavu Town in responsible management of their waste, but the presence at high levels of pathogenic bacteria in the river water and sediment represents a serious threat to the health of the citizens of Bukavu Town. This health threat is exacerbated by the finding that with the exception of Cip, these pathogenic bacteria show extensive resistance to commonly used antibiotics and indicate that outbreaks of enteric disease may reach a stage where only symptomatic treatment may be offered to patients.

The HMs tolerance tests showed that most bacteria isolated in the Kahwa River were resistant to Cd and Pb and that most bacteria are less tolerant to Cd than to Pb. While this may be interpreted as greater and more effective tolerance mechanisms shown by the test bacteria to Pb, it might also mean that Cd is simply more toxic to bacteria than is Pb. In general, it was observed that growth of tolerant bacteria decreased when the concentration of HMs increased. While elevated HMs concentrations may be lethal to bacteria, it is important to determine whether bacteria subjected to extreme concentrations of HMs in fact die, or whether such an inhospitable environment prompts stasis on the part of the

bacteria until such time as the environment surrounding the bacteria changes to one that is less polluted with HMs.

In light of the above, it was observed that the Kahwa River is polluted by indicator and pathogenic bacteria which showed resistance to both antibiotics and HMs. There is evidence that they may pose a serious heath risk problem to citizens and to aquatic life through infection with such resistant microbes which can, in turn, lead to an increase in the incidence of morbidity and mortality amongst the citizens and the animal life in Bukavu Town.

CHAPTER 5: GENERAL CONCLUSION

The research carried out in the Kahwa River aimed to assess HMs and bacterial pollution of sediment and water. In order to achieve the research objectives, investigations focused on i) the source of pollution; ii) the assessment of Cd and Pb concentration and iii) that of pathogenic bacteria. Research focussing on the pollution source survey of the river was conducted from June to August 2013 and aimed to identify possible sources of bacteria and HMs (Cd and Pb) in the Kahwa River catchment. To achieve this goal, a questionnaire was distributed to Bukavu citizens and their responses to the survey were analysed to determine the type and disposal of waste from garages, factories, healthcare facilities and domestic households.

The results obtained in the research showed that wastewater from garages is discarded wherever mainly convenient and the effluent from the garages may contain Cd and Pb that are likely to pollute the sediment and water in the receiving river. Likewise, domestic wastewater is mainly discharged into canals and may also contain polluting HMs. The vast majority of domestic solid waste is incinerated but this practice may spread Cd and Pb dust into the environment which can then return into surface water. The majority of toilets are pit latrines and when overloaded, particularly in the wet season, these toilets may discharge faeces into the river leading to enteric bacterial contamination of its sediment and water. The solid waste generated in the healthcare facilities is incinerated while much of the wastewater from these facilties is released directly into canals. This practice is of great concern as waste from healthcare facilities constitute the main source of bacteria.

The research carried out to assess Cd and Pb pollution in the Kahwa River successfully used AAS to determine the concentration of HMs such as Cd and Pb in the Kahwa River. Results showed that the sediment and water were extensively polluted by Cd and Pb, well above the standard limits set for drinking water and for sediment. Pollution of the Kahwa River by these HMs may have a great impact on all life in the river and in Lake Kivu.

The study also aimed to isolate and identify bacteria from the rive r and to study bacterial resistance to selected antibiotics and tolerance to HMs such as Cd

and Pb. In the process, the samples were collected and analysed for indicator and pathogenic bacterial to show that the river was polluted by both indicator and pathogenic bacteria. These bacteria were extensively resistant to antibiotics and tolerant to HMs. Least resistance was shown to Cip compared to the other antibiotics and all the bacterial strains tested were resistant to Amp. Likewise, tests to determine tolerance to HMs were performed on bacteria isolated from the Kahwa River and showed that all the bacterial strains tested were tolerant to Cd and Pb. In general, lower tolerance to both antibiotics and HMs was shown in the bacteria isolated from water samples compared to the bacteria isolated from the river sediment.

While this increase in resistance may result from the impact of an increase in concentration of both antibiotics as well as HMs accumulating in the sediment from the water, the exciting possibility of the presence of biofilm-like populations of bacteria present in sediment must also be considered. Such colonies of bacteria may dynamically cooperate within the sediment to minimize the impact of antibacterial pollutants such as antibiotics and HMs by increasing mutual transfer of genetic material encoding resistance genes.

The current research should be extended in a biotechnology direction whereby the study of dynamic microbial defence mechanisms such as resistance gene transfer as well as biofilm populations may be translated into practical means whereby we can purify water following the removal from the water of HMs, antibiotics, hormones and a variety of compounds that pollute the water.

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APPENDICES

Appendix A

1. Surveys used in study

1.1. Questionnaire intended for families

Introduction

Dear all,

Please would you fill this questionnaire that is intended for research in order to contribute to building a safe environment and thus to protect our health and that of the people around us. Feel free to respond to the questions as your answers will help to maintain our environment.

I pleased you to tick in the box which corresponds to the best answer

I. Identification

1. Age:
2. Sex: Male ☐

   Female ☐
3. District: ……
4. Avenue: ……..
5. Date: …….

II. Questions

1. Does your family produce the following waste materials?

a. Batteries Yes ☐ No ☐

b. Cooking oil Yes ☐ No ☐

c. Part of food Yes ☐ No ☐

d. Plastics Yes ☐ No ☐

e. Glasses Yes ☐ No ☐

f. Wastewater Yes ☐ No ☐

2. Does your family dispose of garbage cans?

a. Yes ☐

b. No ☐

3. If yes, which type of garbage can does your family dispose?

a. Sack ☐

b. Plastic containers ☐

c. Metallic containers ☐

4. If no, where does it release the waste?

a. Canals ☐

b. Surface water ☐

c. Ground ☐

d. Garden ☐

5. Does your family empty the garbage can regularly?

a) Yes ☐

b) No ☐

6. If yes, where does it empty the garbage can?

a) Canals ☐

b) Surface water ☐

c) Ground ☐

d) Garden ☐

e) Landfills ☐

7. Do the landfills exist in your avenues?

a) Yes ☐

b) No ☐

8. If yes, do the landfills emptied regularly?

c) Yes ☐

d) No ☐

9. Does the waste stored in the landfill undergo the following treatment?

a) Incineration ☐

b) Composting ☐

c) Other means ☐

10. Which of the following source of energy does your family use?

a. Battery ☐

b. Generator ☐

c. Electricity ☐

d. Woody fire ☐

11. Does it dispose of a toilet?

a. Yes ☐

b. No ☐

12. Which kind of toilet does it use?

a. Pit latrine ☐

b. Latrine connected to a canal ☐

c. Toilet connected to surface water ☐

d. Toilet connected to a septic hole ☐

13. Does the toilet waste treated before being released in the environment?

a) Yes ☐

b) No ☐

14. Where does your family release the wastewater?

a) Canals ☐

b) Surface water ☐

c) Ground ☐

15. Is the wastewater treated before being discharged in the environment?

a) Yes ☐

b) No ☐

1.2. Questionnaire intended for hospitals and healthcare centres

Introduction

Dear all,

Please would you fill this questionnaire that is intended for research in order to contribute to building a safe environment and thus to protect our health and that of the people around us. Feel free to respond to the questions as your answers will help to maintain our environment.

You are not obliged to write your name and even your telephone number.

I. Identification

1. Name: ……..

- Age:……

- Sex: male ☐ Female ☐

2. Hospital position……..…

3. The name of the healthcare……..
4. Avenue: ……..
5. District: ……

II. Questions

Tick off or fill the answer in boxes below

1. Does your healthcare host patients?

a) Yes ☐

b) No ☐

2. How many beds does your healthcare have? ☐
3. What is the average number of beds occupied per day? ☐
4. Are all waste containers kept closed except when adding disposed waste?

a) Yes ☐

b) No ☐

5. Does your hospital have a centralized collection area for the waste?

a) Yes ☐

b) No ☐

6. Do staffs who handle waste receive waste management training?

a) Yes ☐

b) No ☐

7. Does the healthcare has a spill plan

a) Yes ☐

b) No ☐

8. Has your facility worked to reduce, eliminate, and to recycle toxic chemicals whenever possible?

a) Yes ☐

b) No ☐

9. Does your healthcare properly store waste in appropriate containers that prevent release of waste into the environment?

a) Yes ☐

b) No ☐

Tick off the best answer(s) in the questions below

10. Does your hospital organize the following services?

a. Pediatric Yes ☐ No ☐

b. Surgery Yes ☐ No ☐

c. Radiography Yes ☐ No ☐

d. Gynecology Yes ☐ No ☐

e. Nephrology Yes ☐ No ☐

11. Does your hospital generate the following waste?

a. Batteries Yes ☐ No ☐

b. Antibiotic residue Yes ☐ No ☐

c. Blood Yes ☐ No ☐

d. Syringe Yes ☐ No ☐

e. Alcohol Yes ☐ No ☐

f. Acids Yes ☐ No ☐

g. Disinfectants Yes ☐ No ☐

h. Dyes Yes ☐ No ☐

i. Stain Yes ☐ No ☐

j. Faecal matter Yes ☐ No ☐

k. Urine Yes ☐ No ☐

12. Does it recycle any of the following wastes?

a. Paper Yes ☐ No ☐

b. Cooking oil Yes ☐ No ☐

c. Glass Yes ☐ No ☐

d. Pallets Yes ☐ No ☐

e. Toner cartridges Yes ☐ No ☐

f. Pharmaceutical Yes ☐ No ☐

g. Plastics Yes ☐ No ☐

13. Does the healthcare stores solid waste?

a) Yes ☐

b) No ☐

1. Where does it store solid waste?

a. Plastics sack Yes ☐ No ☐
b. Hole Yes ☐ No ☐

2. Where does the healthcare discharge wastewater?

a. Sewer Yes ☐ No ☐
b. Septic hole Yes ☐ No ☐
c. River Yes ☐ No ☐
d. Canals Yes ☐ No ☐
e. Ground Yes ☐ No ☐

3. Does your healthcare disinfect the wastewater before being discharged in the environment?

a. Yes ☐
b. No ☐

4. Does your healthcare use the following source of energy?

a. Batteries Yes ☐ No ☐
b. Solar energy Yes ☐ No ☐
c. Electricity Yes ☐ No ☐
d. Generator-based gas oil Yes ☐ No ☐

5. Which of the following means is applied in solid waste management?

a. Incineration off site Yes ☐ No ☐
b. Incineration on site Yes ☐ No ☐
c. Burying Yes ☐ No ☐

1.3. Questionnaire designed for garages

Introduction

Dear all,

Please would you fill this questionnaire that is intended for research in order to contribute to building a safe environment and thus to protect our health and that of the people around us. Feel free to respond to the questions as your answers will help to maintain our environment.

You are not obliged to write your name and even your telephone number.

I. Identification

1. Name: ……..
- Age:
- Sex: male ☐ Female ☐
2. Garage position:.....
3. The name of the garage:…….
4. Avenue: ……..
5. District: ……

II. Questions

Tick off the following answers

1. Does your garage perform the following activities?

a. Welding Yes ☐ No ☐
b. Painting Yes ☐ No ☐
c. Motor revision oil Yes ☐ No ☐
d. Washing car Yes ☐ No ☐

2. Does it produce the following waste?

a. Used oil motor Yes ☐ No ☐
b. Dye Yes ☐ No ☐
c. Metals Yes ☐ No ☐
d. Used tire Yes ☐ No ☐
e. Papers Yes ☐ No ☐
f. Wastewater Yes ☐ No ☐
g. Old and useless battery Yes ☐ No ☐

3. Does the garage stores the waste?

a. No ☐
b. Yes ☐

4. If yes, does it have a centralized collection area for the waste?

a. Yes ☐
b. No ☐

5. Where does the garage discharge waste?

a. River Yes ☐ No ☐
☐

b. Canal Yes ☐ No ☐

c. Ground Yes ☐ No ☐

6. If no, where does it dicharged?

a. River Yes ☐ No ☐

b. Canal Yes ☐ No ☐

c. Ground Yes ☐ No ☐

7. Does the garage treat the waste before being released in the environment?

a) Yes ☐

b) No ☐

c) Does the garage use the following energy sources?

e. Batteries Yes ☐ No ☐

f. Solar energy Yes ☐ No ☐

g. Electricity Yes ☐ No ☐

h. Generator based gas oil yes ☐ No ☐

Appendix B

1. Data generated from HMs analysis in the wet and dry seasons

1.1. Cd and Pb concentration in sediment and water in the wet seasons

1.1.1. Cd and Pb concentration in water

Table 1: Mean value of Pb and Cd concentration in water (in mg/l)

Sites	Sample	Cd (mg/l)			Pb (mg/l)		
		Sept	Oct	Nov	Sept	Oct	Nov
Site I	S1	0.144	0.241	0.058	0.95	0.309	0.65
	S2	0.349	0.230	0.244	0.349	0.212	0.041
	S3	0.241	0.145	0.250	nd	0.405	0.067
Site II	S1	0.341	0.187	0.386	0.243	0.342	0.356
	S2	0.119	0.401	0.292	0.317	0.411	0.248
	S3	0.226	Nd	0.311	0.214	0.315	0.414
Site III	S1	0.112	0.217	0.118	0.246	0.307	nd
	S2	0.231	0.248	0.256	0.402	0.277	0.309
	S3	0.196	0.306	0.127	0.322	0.335	0.40
WHO (2008)		**0,003 mg/l**			**0,010 mg/l**		

1.1.2. Cd and Pb concentration in the sediment

Table 2: Average Pb and Cd concentration in sediment (in mg/g)

Sites	Sample	Cd (mg/g)			Pb (mg/g)		
		Sept	Oct	Nov	Sept	Oct	Nov
Site I	S1	12.41	76.406	56.181	138.17	241.01	146.57
	S2	22.175	54.381	27.012	244.01	267	nd
	S3	46.06	72.413	37.0	206.910	266.50	279.03
Site II	S1	48.0	nd	18.140	104.12	94.O32	87.35
	S2	62.36	nd	46.125	201.24	185.36	194.49
	S3	12.449	48.220	65.178	370.45	nd	418.27
Site III	S1	54.40	123.34	237.04	196.01	216.44	377.08
	S2	67	554.56	29.41	286.48	59.2	0.405
	S3	98.956	82.25	25.09	170.04	227.67	254.56
Macdonald *et al*. (2008)		**0,0099 mg/g**			**0,358 mg/g**		

nd: not determined

1.2. Cd and Pb concentration in sediment and water in the dry season

1.2.1. Cd and Pb concentration in water

Table 3: Mean value of Pb and Cd concentration in water (in mg/l)

Sites	Sample	Cd			Pb		
		May	**June**	**July**	**May**	**June**	**July**
Site I	**S1**	0.136	0.124	0.20	0.22	0.194	0.074
	S2	0.142	0.111	0.158	0.324	0.247	0.330
	S3	0.019	0.126	0.244	0.5	0.338	0.250
Site II	**S1**	0.068	0.10	nd	0.231	0.422	0.345
	S2	0.112	0.120	0.092	0.425	0.320	0.221
	S3	0.233	0.012	0.41	0.318	0.232	0.614
Site III	**S1**	0.021	0.231	0.214	0.432	0.317	0.239
	S2	0.111	0.127	0.09	0.312	0.275	0.341
	S3	0.018	0.211	0.298	0.256	0.292	0.346
WHO (2008)		**0,003 mg/l**			**0,010 µg/l**		
EPA (2009)		**0,005 mg/l**			**0,015 µg/l**		

1.2.2. Cd and Pb concentration in the sediment

Table 4: Average Pb and Cd concentration in sediment (in mg/g)

Sites	Sample	Cd (mg/ml)			Pb (mg/ml)		
		May	**June**	**July**	**May**	**June**	**July**
Site I	**S1**	23.84	41.O6	127.56	191.O6	269.73	104
	S2	46.01	16.39	83.2	232.01	256.46	337.68
	S3	66.43	76.0	137.07	242.16	nd	269.96
Site II	**S1**	37.40	17.02	46.87	122.06	142.16	132.48
	S2	101.12	88.07	122.43	227.17	nd	234
	S3	56.24	59.20	44.32	369.05	nd	nd
Site III	**S1**	63.19	nd	86.22	57.30	94.08	58.67
	S2	nd	69.08	90.25	49.52	123	72.44
	S3	554.56	227.44	214.97	269.76	191.05	125.18
Macdonald *et al*. (2000)		**0,0099 mg/g**			**0,358 mg/g**		

nd: not determined

Appendix C

1. Data generated from bacterial analysis

1.1. Data generated from indicator bacteria analysis

1.1.1. Indicator bacteria isolated in the wet season

1.1.1.1. Indicator bacteria isolated in water

Table 5: The average number of indicator bacteria in water

Sites	Sampl	CfU/ml											
		Escherichia coli			***Enterococci***			**Fecal coliform**			**Total coliform**		
		Sept	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**
Site I	**S1**	4	0	3	4	4	2	3	0	0	3	2	3
	S2	3	3	2	3	2	3	4	3	3	2	0	0
	S3	4	2	0	2	2	3	3	3	0	4	3	3
Site II	**S1**	3	3	0	4	2	3	3	0	2	2	3	3
	S2	3	3	4	5	5	3	4	3	3	4	3	4
	S3	4	3	3	6	4	3	3	2	2	3	2	4
Site III	**S1**	3	2	0	3	4	0	3	2	3	3	2	2
	S2	7	3	4	2	5	4	0	0	2	3	4	2
	S3	2	0	0	4	2	2	3	3	2	4	3	3

1.1.1.2. Indicator bacteria isolated in the sediment

Table 6: The average number of indicator bacteria in the sediment

Sites	Sampl	CfU/ml											
		Escherichia coli			***Enterococci***			**Fecal coliform**			**Total coliform**		
		Sept	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**
Site I	**S1**	4	2	2	6	4	4	4	3	0	4	5	3
	S2	5	0	4	7	5	3	4	2	3	5	4	3
	S3	4	2	3	4	4	5	6	3	4	4	3	2
Site II	**S1**	3	3	7	5	0	6	4	0	6	2	8	3
	S2	5	4	4	7	5	3	4	6	6	4	10	4
	S3	4	6	4	8	6	4	5	3	6	4	2	5
Site III	**S1**	3	6	4	5	4	4	3	5	5	8	4	7
	S2	6	7	4	11	5	3	0	4	3	5	4	5
	S3	3	4	0	4	7	0	4	4	2	4	2	3

1.1.2. Indicator bacteria isolated in the dry season

1.1.2.1. Indicator bacteria isolated in water

Table 7: The average number of indicator bacteria in water

Sites	Col	CfU/ml											
		Escherichia coli			***Enterococci***			**Fecal coliform**			**Total coliform**		
		May	**June**	**July**	**May**	**June**	**July**	**May**	**June**	**July**	**May**	**June**	**July**
Site I	S1	2	1	0	4	4	2	3	0	2	3	2	3
	S2	3	2	3	4	3	1	4	3	5	2	0	0
	S3	2	2	4	3	6	3	3	3	0	4	3	3
Site II	S1	3	3	1	3	2	3	3	1	2	2	3	3
	S2	3	3	4	2	4	7	2	3	3	3	4	0
	S3	3	0	3	4	2	2	3	4	3	3	3	4
Site III	S1	3	2	0	3	4	0	3	2	3	3	0	2
	S2	7	3	4	2	5	4	0	3	4	3	6	1
	S3	2	0	0	4	2	2	4	1	1	3	2	5

1.1.2.2. Indicator bacteria isolated in the sediment

Table 8: The average number of indicator bacteria in the sediment

Sites	CfU/ml											
	Escherichia coli			***Enterococci***			**Fecal coliform**			**Total coliform**		
	May	**June**	**July**	**May**	**June**	**July**	**May**	**June**	**July**	**May**	**June**	**july**
Site I	2	3	3	6	5	4	5	0	0	4	2	3
	0	0	4	4	3	0	2	3	3	1	3	3
	3	3	2	6	5	5	4	4	2	4	3	1
Site II	4	1	2	4	3	6	2	3	8	2	0	3
	5	3	4	4	3	3	3	6	4	4	2	5
	4	3	1	6	7	4	1	3	6	1	2	1
Site III	2	3	4	5	4	4	3	3	1	4	4	2
	4	2	0	4	5	4	0	4	3	3	3	4
	5	0	1	3	3	2	4	4	2	5	2	3

1.2. Data generated from pathogenic bacteria analysis

1.2.1. Pathogenic bacteria in sediment and water in the wet season

1.2.1.1. Pathogenic bacteria isolated in water

Table 9: Average number of pathogenic bacteria in water

Sites	Sample	Number of positive plates								
		Salmonella spp.			***Shigella spp.***			***Vibrio spp.***		
		Sept	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**	**Sept**	**Oct**	**Nov**
Site I	S1	2	1	0	0	0	1	1	3	2
	S2	3	2	1	0	2	1	2	3	3
	S3	1	2	2	0	0	0	2	2	1
Site II	S1	3	3	1	3	1	0	2	5	3
	S2	2	0	0	0	0	0	3	3	4
	S3	1	1	0	0	2	2	4	5	5
Site III	S1	2	2	0	1	0	0	3	3	2
	S2	0	3	1	0	0	0	2	4	5
	S3	3	4	0	2	2	0	4	5	5

1.2.1.2. Bacteria analysis in the sediment

Table 10: Average number of pathogenic bacteria in sediment

Sites	Sample	Number of positive plates (n=5)								
		Salmonella spp.			*Shigella spp*.			*Vibrio spp*.		
		Sept	Oct	Nov	Sept	Oct	Nov	Sept	Oct	Nov
Site I	S1	0	2	1	1	0	0	0	1	2
	S2	3	2	1	0	0	0	4	0	0
	S3	1	2	4	1	1	0	4	2	0
Site II	S1	3	3	1	3	1	0	2	5	3
	S2	4	1	2	0	1	0	4	4	0
	S3	1	1	0	0	2	2	4	5	3
Site III	S1	2	3	3	3	2	0	3	4	3
	S2	0	2	1	0	2	0	5	5	0
	S3	3	5	4	3	2	2	5	5	5

1.2.2. Pathogenic bacteria in sediment and water in the dry season

1.2.2.1. Pathogenic bacteria isolated in water

Table 11: Average number of pathogenic bacteria in water

Sites	Sample	Number of positive plates								
		Salmonella spp.			*Shigella spp*.			*Vibrio spp*.		
		May	June	June	May	June	July	May	June	July
Site I	S1	1	3	3	0	0	1	3	4	4
	S2	0	2	1	1	1	3	5	3	3
	S3	1	0	3	0	2	2	3	4	5
Site II	S1	3	4	1	2	3	3	4	3	3
	S2	2	3	4	0	0	3	4	5	5
	S3	2	3	0	0	0	3	4	4	5
Site III	S1	3	3	0	1	0	0	0	2	2
	S2	0	0	1	1	2	1	4	5	4
	S3	2	2	1	0	0	2	3	4	2

1.2.2.2. Pathogenic bacteria isolated in the sediment

Table 12: Average number of pathogenic bacteria in sediment

Sites	Number of positive plates								
	Salmonella spp.			*Shigella spp*.			*Vibrio spp*.		
	May	June	July	May	June	July	May	June	July
Site I	3	3	2	0	4	4	5	2	2
	4	3	3	2	0	3	3	3	5
	0	3	1	0	3	3	3	4	5
Site II	2	3	3	3	1	2	4	5	3
	3	3	2	0	0	0	4	4	5
	0	3	3	1	2	0	3	0	0
Site III	0	1	1	0	0	0	2	1	4
	2	2	0	0	1	1	3	4	2
	3	3	4	1	1	2	3	5	5

1.3. Identification of bacteria isolated the wet and dry seasons

1.3.1. Identification of pathogenic bacteria isolated in the wet season

1.3.1.1. Identification of pathogenic bacteria isolated in water

Table 13: Average number of pathogenic bacteria in water

Sites	Sample	Number of positive test tubes (n=5)											
		S. typhi			*S. paratyphi*			*Shigella spp*			*Vibrio cholerae*		
		Sept	Oct	Nov	Sept	Oct	Nov	Sept	Oct	Nov	Sept	Oct	Nov
Site I	S1	nd	Nd	0	1	Nd	0	0	0	2	3	2	4
	S2	2	2	1	3	3	0	0	2	nd	3	0	4
	S3	0	Nd	2	2	3	1	0	0	0	0	3	0
Site II	S1	0	1	0	2	Nd	2	3	nd	0	3	4	2
	S2	1	2	ind	1	1	0	0	1	0	1	1	3
	S3	3	Nd	0	nd	1	0	0	1	nd	2	4	3
Site III	S1	1	Nd	0	nd	3	0	nd	0	0	4	2	2
	S2	0	1	nd	0	3	1	0	0	0	3	5	4
	S3	2	Nd	0	3	2	0	1	3	0	5	2	2

nd: not defined. This means that the pathogen tested was either positive to KIA or to TSI but not to the two tests at the same time.

1.3.1.2. Identification of pathogenic bacteria isolated in the sediment

Table 14: The average number of pathogenic bacteria in sediment

Sites	Sample	Number of positive test tubes											
		S. typhi			*S. paratyphi*			*Shigella spp*			*Vibrio cholerae*		
		Sept	Oct	Nov	Sept	Oct	Nov	Sept	Oct	Nov	Sept	Oct	Nov
Site I	S1	0	1	2	0	3	nd	Nd	0	0	0	2	3
	S2	nd	1	nd	2	1	nd	0	0	0	2	0	0
	S3	nd	nd	nd	1	Nd	2	1	2	0	3	3	0
Site II	S1	2	nd	nd	2	Nd	2	2	nd	0	2	1	2
	S2	1	nd	nd	2	1	nd	0	2	0	2	1	2
	S3	2	nd	0	3	1	0	Nd	nd	nd	3	3	3
Site III	S1	nd	nd	2	1	Nd	nd	Nd	1	0	3	3	2
	S2	0	1	1	0	3	nd	0	2	0	2	2	2
	S3	1	nd	1	2	2	1	1	nd	nd	3	2	3

1.3.2. Identification of pathogenic bacteria isolated the dry season

1.3.2.1. Identification of pathogenic bacteria isolated in water

Table 15: Average number of pathogenic bacteria in water

Sites	Sample	Number of positive test tubes (n=5)											
		S. typhi			*S. paratyphi*			*Shigella dysenteriae*			*Vibrio cholerae*		
		May	June	Jul	May	Jun	July	May	June	July	May	June	July
Site I	S1	1	1	0	0	1	1	0	2	1	2	3	3
	S2	1	ind	0	1	1	nd	0	1	nd	2	2	1
	S3	0	1	3	2	nd	nd	0	1	nd	2	2	2
Site II	S1	1	2	2	2	3	1	1	Nd	0	3	3	2
	S2	1	0	0	1	2	2	0	1	1	3	2	3
	S3	0	1	0	0	1	2	1	0	nd	3	1	2
Site III	S1	0	0	0	nd	3	2	nd	1	0	1	3	1
	S2	0	0	1	0	1	2	1	0	0	2	2	1
	S3	1	2	0	1	1	0	0	2	2	3	3	1

1.3.2.2. Identification of pathogenic bacteria isolated in the sediment

Table 16: The average number of pathogenic bacteria in sediment

Sites	Sample	Number of positive test tubes (n=5)											
		S. typhi			*S. paratyphi*			*Shigella dysenteriae*			*Vibrio cholerae*		
		May	June	July	May	June	July	May	June	July	May	June	July
Site I	S1	1	0	0	3	1	nd	0	2	0	2	2	2
	S2	0	2	nd	1	1	0	0	0	1	2	0	1
	S3	0	nd	0	0	2	1	1	nd	0	2	3	3
Site II	S1	0	1	0	0	nd	1	0	2	2	1	1	2
	S2	0	0	1	0	1	1	0	2	0	2	2	3
	S3	2	1	0	nd	1	3	1	1	0	2	2	4
Site III	S1	0	1	0	1	1	0	0	0	2	3	3	1
	S2	0	0	nd	0	0	1	0	0	0	1	3	4
	S3	2	0	0	2	2	3	1	0	0	5	3	2

1.4. Antibiotic susceptibility test

1.4.1. Antibiotic susceptibility test on bacteria isolated in the wet season

1.4.1.1. Antibiotic susceptibility test on bacteria isolated in water

Table 17: Relative growth in the presence of antibiotics of bacteria from water

Bateria	**Indic.**	**Site I**					**Site II**					**Site III**				
		Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp
Vibrio cholerae	Suscep	3	1	3	0	1	3	0	2	0	0	3	0	3	0	0
	Interm.	0	0	0	2	0	0	2	1	0	1	0	2	0	2	0
	Resist.	0	2	0	1	1	0	1	0	3	2	0	1	0	1	3
S. typhi	Suscep	3	1	1	0	0	0	0	3	0	0	0	1	0	0	0
	Interm.	0	2	2	1	2	3	2	0	2	3	1	1	3	3	2
	Resist.	0	0	0	2	1	0	1	0	1	0	2	1	0	0	1
S. paratyp	Suscep	0	2	3	0	0	0	0	0	0	0	0	1	0	0	0
	Interm	3	1	0	3	2	2	2	3	3	0	2	2	2	1	2
	Resist.	0	0	0	0	1	1	1	0	0	3	1	0	1	2	1
Shigella dysenteriae	Suscep	3	3	2	0	0	3	2	1	2	0	0	0	0	0	0
	Interm	0	0	1	2	2	0	0	1	0	3	2	2	2	0	3
	Resist	0	0	0	1	1	0	1	1	0	0	1	0	1	3	0
E coli	Suscep	3	2	0	3	0	3	0	2	3	0	3	2	2	3	0
	Interm	0	0	2	0	1	0	2	1	0	2	0	0	0	0	2
	Resist	0	1	1	0	2	0	1	0	1	1	0	1	1	0	1
Entero-cocci	Suscep	3	0	3	0	3	3	0	0	0	3	3	1	1	0	2
	Interm	0	3	0	2	0	0	2	2	3	0	0	1	0	2	0
	Resist	0	0	0	1	0	0	1	0	0	0	0	1	2	1	1
Faecal coliforms	Suscep	3	0	1	0	0	3	0	3	0	0	3	3	3	0	2
	Interm	0	0	0	2	1	0	1	0	2	3	0	0	0	2	0
	resist	0	3	2	1	2	0	2	0	1	0	0	0	0	1	1
Total coliforms	suscep	3	3	2	0	1	3	0	0	0	1	3	2	3	3	0
	Interm	0	0	0	2	0	0	3	2	3	2	0	0	0	0	1
	Resist	0	0	1	1	2	0	0	1	0	0	0	1	0	0	2

1.4.1.2. Antibiotic susceptibility test on bacteria isolated in the sediment

Table 18: Relative growth in the presence of antibiotics of bacteria from sediment

Bacteria	**Indic.**	**Site I**					**Site II**					**Site III**				
		Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp
Vibrio ch	Suscep	3	0	0	1	0	3	0	0	1	0	3	0	2	0	0
	Interm.	0	0	1	1	0	0	0	0	1	1	0	2	0	2	1
	Resist.	0	3	2	1	3	0	3	3	1	2	0	1	1	1	2
S. typhi	Suscep	3	0	0	0	3	0	0	0	0	0	2	0	0	0	3
	Interm.	0	0	1	2	0	1	0	2	1	1	0	2	3	1	0
	Resist.	0	3	2	1	0	2	3	1	2	3	1	1	0	2	0
S. paratypi	Suscep	2	0	3	2	0	0	0	0	0	0	3	0	0	1	0
	Interm	0	0	0	0	0	2	1	0	2	1	0	3	1	1	2
	Resist.	1	3	0	1	3	1	2	3	1	2	0	0	2	1	1
Shigella	Suscep	3	3	0	1	0	1	0	0	0	2	3	0	2	0	0
	Interm	0	0	2	2	1	2	2	3	0	1	0	1	1	3	2
	Resist	0	0	1	0	2	0	1	0	3	0	0	2	0	0	1
E coli	Suscep	3	1	0	2	3	2	0	3	2	0	3	0	0	0	0
	Interm	0	1	0	0	0	0	1	0	1	2	0	3	2	2	0
	Resist	0	1	3	1	0	1	2	0	0	1	0	0	1	0	3
Entero-cocci	Suscep	3	2	2	0	0	0	0	0	0	0	3	0	0	1	2
	Interm	0	1	1	2	0	3	2	0	2	0	0	1	1	0	0
	Resist	0	0	0	1	3	0	1	3	1	3	0	2	2	1	1
Faec colif	Suscep	3	1	2	2	2	3	0	3	0	3	0	0	3	1	0
	Interm	0	0	0	1	0	0	2	0	3	0	1	2	0	2	0
	resist	0	2	1	0	1	0	1	0	0	0	2	1	0	0	3
Total colif	suscep	3	2	1	2	0	3	2	0	2	0	0	0	1	1	0
	Interm	0	0	1	0	2	0	0	0	0	1	1	3	0	1	2
	Resist	0	0	1	1	1	0	1	3	1	2	2	0	2	1	1

1.4.2. Antibiotic susceptibility test on bacteria isolated in the dry season

1.4.2.1. Antibiotic susceptibility test on bacteria isolated in water

Table 19: Relative growth in the presence of antibiotics of bacteria from water

Bateria	**Indic.**	**Site I**					**Site II**					**Site III**				
		Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp
Vibrio ch	Suscep	2	2	0	2	0	2	1	0	0	0	1	1	2	2	1
	Interm.	1	0	1	0	1	0	0	1	0	0	1	0	0	0	0
	Resist.	0	1	2	1	2	1	2	2	0	3	1	2	1	1	2
S. typhi	Suscep	2	2	0	2	3	0	2	0	2	0	1	0	0	2	0
	Interm.	1	0	1	0	1	2	0	2	0	0	2	1	1	0	0
	Resist.	0	1	2	1	2	1	1	1	1	3	0	2	2	1	3
S. paratyp	Suscep	0	0	1	0	2	2	1	1	0	0	1	2	0	1	0
	Interm	1	0	1	2	0	0	1	0	1	2	2	1	0	0	2
	Resist.	2	3	1	1	1	1	1	2	2	1	0	0	3	2	1
Shigella	Suscep	3	2	1	1	0	3	1	1	2	1	0	0	2	3	0
	Interm	0	0	0	1	0	0	0	0	0	0	2	2	0	0	0
	Resist	0	1	2	1	3	0	2	2	1	2	1	1	1	0	3
E coli	Suscep	2	2	0	3	0	2	0	2	2	0	3	1	0	3	0
	Interm	0	0	1	0	0	1	1	0	0	0	0	2	0	0	0
	Resist	1	1	2	0	3	0	2	1	1	3	0	0	3	0	3
Enteroco	Suscep	3	3	2	0	0	3	0	1	1	1	3	2	1	2	0
	Interm	0	0	0	1	2	0	2	1	2	0	0	1	2	0	2
	Resist	0	0	1	2	1	0	1	1	0	2	0	0	0	1	1
Faec colif	Suscep	1	3	2	2	0	0	3	2	0	1	3	1	3	2	0
	Interm	2	0	0	0	1	1	0	1	2	0	0	2	0	0	2
	resist	0	0	1	1	2	2	0	0	1	2	0	0	1	1	1
Total colif	suscep	3	1	2	0	0	3	1	1	0	1	2	2	3	3	0
	Interm	0	1	0	1	2	0	2	1	1	1	1	0	0	0	1
	Resist	0	1	1	2	1	0	0	1	2	1	0	1	0	0	2

1.4.2.2. Antibiotic susceptibility test on bacteria isolated in the sediment

Table 20: Relative growth in the presence of antibiotics of bacteria from sediment

Bacteria	Indic.	Site I					Site II					Site III				
		Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp	Cip	Te	Sxt	C	Amp
Vibrio ch	Suscep	2	0	0	2	0	1	0	2	1	0	2	0	1	2	0
	Interm.	0	1	1	0	0	0	1	0	0	1	0	2	1	0	1
	Resist.	1	2	1	1	3	2	2	1	2	2	1	1	1	1	2
S. typhi	Suscep	3	0	0	2	2	1	0	0	0	0	1	1	0	1	1
	Interm.	0	0	1	0	0	1	1	2	2	1	0	1	2	1	0
	Resist.	0	3	2	1	1	1	2	1	1	2	2	1	1	1	2
S. paratyp	Suscep	0	3	0	2	0	2	0	0	0	0	0	0	1	0	0
	Interm	1	1	3	0	0	0	2	2	1	1	3	2	1	1	0
	Resist.	2	2	0	1	3	1	1	1	2	2	0	1	1	2	3
Shigella spp.	Suscep	3	1	0	0	0	0	0	0	0	0	2	0	1	0	0
	Interm	0	0	2	2	0	2	2	0	1	2	1	1	1	1	2
	Resist	0	2	1	1	3	1	1	3	2	1	1	2	1	2	1
E coli	Suscep	3	3	2	3	2	3	1	3	0	1	3	1	0	0	0
	Interm	0	0	0	0	0	0	1	0	1	0	0	0	2	2	2
	Resist	0	0	1	0	1	0	1	0	2	2	0	2	1	1	1
Entero-cocci	Suscep	3	1	2	0	0	2	1	0	0	0	2	0	2	0	0
	Interm	0	1	0	1	1	1	0	2	1	2	0	1	1	1	0
	Resist	0	1	1	2	2	0	2	1	2	1	1	2	0	2	3
Faec colif	Suscep	3	3	1	0	0	3	0	3	1	0	3	2	0	1	1
	Interm	0	0	0	3	1	0	2	0	2	1	0	1	2	2	0
	resist	0	0	2	0	2	0	1	0	0	2	0	0	1	0	2
Total colif	suscep	3	0	3	0	0	3	0	0	0	1	3	1	0	0	0
	Interm	0	1	0	3	1	0	2	2	3	0	0	2	2	2	0
	Resist	0	2	0	0	2	0	1	1	0	2	0	0	1	1	3

1.5. Heavy metal susceptibility

1.5.1. Cd and Pb susceptibility on bacteria isolated in the wet season

1.5.1.1. Cd susceptibility on bacteria isolated in water

Tale 21: Average number of Cd-tolerant bacteria isolated in water

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	1	0	0	0	0	2	2	0	0	0	0	1	0	0	0
	Susc	2	3	3	3	3	1	1	3	3	3	3	2	3	3	3
S. typ	Resis	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0
	Susc	3	3	3	3	3	3	1	1	3	3	3	3	3	3	3
S. par	Resis	1	0	0	0	0	0	2	0	0	0	0	1	0	0	0
	Susc	2	3	3	3	3	3	1	3	3	3	3	2	3	3	3
Shigel	Resis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Susc	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
E coli	Resis	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Susc	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3
Entero	Resis	1	1	0	0	0	2	0	1	0	0	1	0	0	0	0
	Susc	2	2	3	3	3	1	3	2	3	3	2	3	3	3	3
F colif	Resis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Susc	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
T. colif	Resis	0	0	0	0	0	2	1	0	0	0	1	0	0	0	0
	Susc	3	3	3	3	3	1	2	3	3	3	2	3	3	3	3

1.5.1.2. Cd susceptibility on bacteria isolated in the sediment

Table 22: Average number of Cd-tolerant bacteria in the sediment

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	1	0	0	0	0	1	2	0	0	0	1	0	0	0	0
	Susc	2	3	3	3	3	2	1	3	3	3	2	3	3	3	3
S. typ	Resis	2	1	0	0	0	1	1	2	0	0	1	1	0	0	0
	Susc	1	2	3	3	3	2	2	1	3	3	2	2	3	3	3
S. par	Resis	0	0	0	0	0	2	1	0	0	0	1	3	0	0	0
	Susc	3	3	3	3	3	1	2	3	3	3	2	0	3	3	3
Shigel	Resis	1	0	0	0	0	2	2	0	0	0	0	2	0	0	0
	Susc	2	3	3	3	3	1	1	3	3	3	0	1	3	3	3
E coli	Resis	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0
	Susc	3	3	3	3	3	3	2	3	3	3	1	3	3	3	3
Enter	Resis	1	0	0	0	0	2	1	1	0	0	0	1	0	0	0
	Susc	2	3	3	3	3	1	2	2	3	3	0	2	3	3	3
F colif	Resis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Susc	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3
T. coli	Resis	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0
	Susc	3	2	3	3	3	2	1	2	3	3	3	2	3	3	3

1.5.1.3. . Pb susceptibility on bacteria isolated in water

Table 23: Average number of Pb-tolerant bacteria in water

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	2	2	1	1	2	3	2	3	2	1	3	3	1	2	1
	Susc	1	1	2	2	1	0	1	0	1	2	0	0	2	1	2
S. typ	Resis	3	3	2	1	1	1	3	2	1	1	3	2	1	1	1
	Susc	0	0	1	2	2	2	0	1	2	2	0	1	2	2	2
S. par	Resis	2	1	2	1	1	3	2	2	2	0	3	3	1	2	1
	Susc	1	2	1	2	2	0	1	1	1	3	0	0	2	1	2
Shigel	Resis	2	2	1	0	0	3	2	1	1	2	3	3	2	2	1
	Susc	1	1	2	3	3	0	1	2	2	1	0	0	1	1	2
E coli	Resis	3	2	0	0	0	2	1	1	1	0	2	3	1	1	0
	Susc	0	1	3	3	3	1	2	2	2	3	1	0	2	2	3
Enter	Resis	3	2	0	0	1	2	3	1	1	0	1	3	2	1	1
	Susc	0	1	3	3	2	1	0	2	2	3	2	0	1	2	2
F colif	Resis	3	1	1	1	0	2	2	1	1	1	3	3	0	2	1
	Susc	0	2	2	2	3	1	1	2	2	2	0	0	3	1	2
T. coli	Resis	3	2	0	0	0	1	2	1	1	0	1	2	1	1	0
	Susc	0	1	1	3	3	2	1	2	2	3	2	1	2	2	3

1.5.1.4 Pb susceptibility on bacteria isolated in the sediment

Table 24: Average number of Pb-tolerant bacteria in the sediment

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	3	3	1	1	0	3	3	3	1	1	3	3	3	1	0
	Susc	0	0	2	2	3	0	0	0	2	2	0	0	0	2	3
S. typ	Resis	3	3	3	1	0	3	3	2	1	1	3	3	2	0	0
	Susc	0	0	0	2	3	0	0	1	2	2	0	0	1	3	3
S. par	Resis	3	3	1	1	1	3	2	2	1	1	3	3	1	1	0
	Susc	0	0	2	2	2	0	1	1	2	2	0	0	2	2	3
Shigel	Resis	3	2	2	1	0	3	2	3	0	0	3	2	1	1	1
	Susc	0	1	1	2	3	0	1	0	3	3	0	1	2	2	2
E coli	Resis	2	1	2	0	0	3	3	1	1	0	3	1	2	0	0
	Susc	1	2	1	3	3	0	0	2	2	3	0	2	1	3	3
Enter	Resis	3	2	3	2	1	2	1	1	2	2	2	3	1	2	0
	Susc	0	1	0	1	2	1	2	2	1	1	1	0	2	1	3
F colif	Resis	2	2	1	0	0	3	1	3	0	1	3	2	1	0	0
	Susc	1	1	2	3	3	0	2	0	3	2	0	1	2	3	3
T. coli	Resis	3	2	2	1	0	3	3	3	0	0	1	3	2	1	0
	Susc	0	1	1	2	3	0	0	0	3	3	2	0	1	2	3

1.5.2. Cd and Pb susceptibility on bacteria isolated in the dry season

1.5.2.1. Cd susceptibility on bacteria isolated in water

Table 25: Average number of Cd-tolerant bacteria isolated in water

Bateri	**Indic.**	**Site I**					**Site II**					**Site III**				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	2	0	0	0	0	1	1	0	0	0	2	1	0	0	0
	Susc	1	3	3	3	3	2	2	3	1	3	1	2	3	3	3
S. typ	Resis	2	0	0	0	0	1	2	1	0	0	2	1	0	0	0
	Susc	1	3	3	3	3	2	1	2	3	3	1	2	3	3	3
S. par	Resis	1	0	0	0	0	1	2	0	0	0	0	1	0	0	0
	Susc	2	3	3	3	3	2	1	3	3	3	0	2	3	3	3
Shigel	Resis	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0
	Susc	3	3	3	3	3	3	2	3	3	3	1	3	3	3	3
E coli	Resis	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0
	Susc	3	3	3	3	3	2	2	3	3	3	2	3	3	3	3
Enter	Resis	1	0	0	0	0	0	2	0	0	0	1	0	0	0	0
	Susc	2	3	3	3	3	3	1	3	3	3	2	3	3	3	3
F colif	Resis	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
	Susc	3	3	3	3	3	2	3	3	3	3	3	2	3	3	3
T. coli	Resis	1	0	0	0	2	0	0	0	0	0	1	0	0	0	0
	Susc	2	3	3	3	1	3	3	3	3	3	2	3	3	3	3

1.5.2.2. Cd susceptibility on bacteria isolated in the sediment

Table 26: Average number of Cd-tolerant bacteria in the sediment

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	1	2	1	0	0	3	3	2	2	0	2	3	1	0	0
	Susc	2	1	2	3	3	0	0	1	1	3	1	0	2	3	3
S. typ	Resis	3	2	0	1	0	3	1	0	0	0	2	2	1	0	0
	Susc	0	1	3	2	3	0	2	3	3	3	1	1	2	3	3
S. par	Resis	2	0	0	0	0	1	1	2	0	0	3	3	0	0	0
	Susc	1	3	3	3	3	2	2	1	3	3	0	0	3	3	3
Shigel	Resis	2	0	0	1	0	2	3	0	0	0	3	2	0	0	0
	Susc	1	3	3	2	3	1	0	3	3	3	0	1	3	3	3
E coli	Resis	2	1	0	0	0	3	1	1	0	0	2	0	0	0	0
	Susc	1	2	3	3	3	0	2	2	3	3	1	3	3	3	3
Enter	Resis	1	1	0	0	0	2	2	1	0	0	3	1	0	0	0
	Susc	2	2	3	3	3	1	1	2	3	3	0	2	3	3	3
F colif	Resis	1	0	0	0	0	1	1	0	0	0	2	1	0	0	0
	Susc	2	3	3	3	3	2	2	3	3	3	1	2	3	3	3
T. coli	Resis	1	0	0	0	0	2	2	1	0	0	1	1	0	0	0
	Susc	2	3	3	3	3	1	1	2	3	3	2	2	3	3	3

1.5.2.1. Pb susceptibility on bacteria isolated in water

Table 27: Average number of Pb-tolerant bacteria in water

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	3	2	2	2	2	3	3	3	1	0	3	3	2	1	1
	Susc	0	1	1	1	1	0	0	0	2	3	0	0	1	3	3
S. typ	Resis	3	3	1	1	0	3	3	2	3	1	3	3	3	0	1
	Susc	0	0	2	2	3	0	0	1	0	2	0	0	0	3	2
S. par	Resis	3	2	2	0	0	3	3	3	3	3	3	3	3	2	3
	Susc	0	1	1	3	3	0	0	0	0	0	0	0	0	1	0
Shigel	Resis	3	3	0	0	0	3	3	1	1	2	3	3	2	0	1
	Susc	0	0	3	3	3	0	0	2	2	1	0	0	1	3	2
E coli	Resis	3	3	0	0	0	3	3	1	0	0	3	3	0	0	0
	Susc	0	0	3	3	3	0	0	2	3	3	0	0	3	3	3
Enter	Resis	3	3	1	0	1	3	3	3	0	0	3	3	0	1	1
	Susc	0	0	2	3	2	0	0	0	3	3	0	0	3	2	2
F colif	Resis	3	3	0	1	0	3	3	1	0	1	3	3	0	2	0
	Susc	0	0	3	2	3	0	0	2	3	2	0	0	3	1	2
T. coli	Resis	3	2	1	0	0	3	3	0	0	0	3	2	1	0	0
	Susc	0	1	2	3	3	0	0	3	3	3	0	1	2	3	3

1.5.2.2 Pb susceptibility on bacteria isolated in the sediment

Table 28: Average number of Pb-tolerant bacteria in the sediment

Bateri	Indic.	Site I					Site II					Site III				
		0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vib ch	Resis	3	3	3	2	2	3	3	3	3	3	3	3	3	3	3
	Susc	0	0	0	1	1	0	0	0	0	0	0	0		0	0
S. typ	Resis	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3
	Susc	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
S. par	Resis	3	3	3	2	1	3	3	3	3	3	3	3	3	3	3
	Susc	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0
Shigel	Resis	3	3	2	1	0	3	3	3	1	1	3	2	3	3	3
	Susc	0	0	1	2	3	0	0	0	2	2	0	1	0	0	0
E coli	Resis	3	3	2	0	0	3	3	0	0	0	3	0	0	0	0
	Susc	0	0	1	3	3	0	0	3	3	3	0	3	3	3	3
Enter	Resis	3	3	3	1	1	3	3	3	3	2	3	3	3	3	3
	Susc	0	0	0	2	2	0	0	0	0	1	0	0	0	0	0
F colif	Resis	3	3	3	1	0	3	3	3	3	1	3	3	3	2	2
	Susc	0	0	0	2	3	0	0	0	0	2	0	0	0	1	1
T. coli	Resis	3	3	1	0	0	3	3	0	0	0	3	2	2	0	0
	Susc	2	0	2	3	3	0	0	3	3	3	0	1	1	3	3

1.6. Effect of HMs on the growth of bacteria

1.6.1. Effect of Cd and Pb on the growth of bacteria isolated the wet season

1.6.1.1. Effect of Cd on the growth of bacteria isolated in water

Table 29: Average number of Cd-tolerant bacteria isolated in water

Bateri Indic.	**Number of positive plates**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	2	1	2	1	0	1	1	1	0	0	2	2	2	1	0
S. typhy	1	3	2	1	2	2	2	2	0	1	2	1	1	1	0
S. pararatyph	3	1	1	2	0	2	3	1	1	0	1	2	1	0	0
Shigella dysenteriae	1	1	1	1	0	3	2	1	0	2	2	2	1	1	0
E coli	1	1	2	0	0	3	1	2	1	0	2	3	1	0	0
Enterococci	3	3	2	1	0	2	3	1	0	0	3	2	1	0	0
Fe coliforms	3	2	0	0	0	2	1	1	0	2	3	2	1	0	0
T. coliforms	2	2	1	0	0	2	1	1	2	0	2	1	1	0	0

1.6.1.2. Effect of Cd on the growth of bacteria isolated in the sediment

Table 30: Average number of Cd-tolerant bacteria in the sediment

Bateri Indic.	**Number of positive plates (n=3)**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	2	2	2	1	0	3	2	1	2	1	2	3	1	1	1
S. typhy	2	1	2	1	1	2	3	2	2	2	3	2	1	2	1
S. pararatyph	2	3	1	2	1	3	3	1	0	1	3	2	1	1	2
Shigella dysenteriae	3	2	1	0	2	2	2	1	0	0	2	1	1	1	0
E coli	3	1	2	0	0	2	2	2	0	0	3	1	0	2	0
Enterococci	2	2	0	1	1	2	2	1	1	2	2	1	2	2	1
Fe coliforms	2	2	1	2	0	3	2	1	1	1	2	2	1	0	1
T. coliforms	3	1	1	2	0	2	2	2	1	0	2	3	1	0	0

1.6.1.3. Effect of Pb on the growth of bacteria isolated in water

Table 31: Average number of Pb-tolerant bacteria in water

Bateri Indic.	**Number of positive plates**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	3	3	1	2	1	3	3	3	2	2	3	3	2	1	2
S. typhy	3	2	2	2	2	3	2	3	3	3	3	2	2	2	2
S. pararatyph	2	3	2	2	1	2	3	3	1	3	3	2	3	1	1
Shigella dysenteriae	3	3	1	1	1	3	2	1	2	1	2	2	1	2	1
E coli	2	3	2	3	0	3	2	2	1	2	2	3	1	0	1
Enterococci	3	2	1	2	2	2	3	2	1	1	3	2	3	1	2
Fe coliforms	2	2	1	1	2	2	1	1	0	2	3	2	1	1	2
T. coliforms	3	2	1	1	1	2	1	1	0	0	2	1	1	2	1

1.6.1.4. Effect of Pb on the growth of bacteria isolated in the sediment

Table 32: Average number of Pb-tolerant bacteria in the sediment

Bateri Indic.	**Number of positive plates (n=3)**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	3	2	3	2	3	3	3	2	3	3	2	3	3	2	3
S. typhy	2	3	3	2	2	3	3	2	3	2	3	2	3	2	2
S. pararatyph	2	3	2	1	2	3	3	3	1	1	3	2	2	3	2
Shigella dysenteriae	3	3	1	2	2	3	2	2	1	0	2	2	0	0	1
E coli	3	2	2	2	1	2	2	2	2	2	3	1	0	1	2
Enterococci	2	2	2	1	3	2	3	1	2	1	2	1	2	1	1
Fe coliforms	2	2	1	0	2	3	2	3	1	1	2	2	1	2	0
T. coliforms	3	3	1	1	1	3	3	2	3	1	2	3	1	2	1

1.6.2. Effect of Cd and Pb on the growth of bacteria in the dry season

1.6.2.1. Effect of Cd on the growth of bacteria isolated in water

Table 34: Average number of Cd-tolerant bacteria isolated in water

Bateri Indic.	**Number of positive plates**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	2	2	1	2	0	1	2	1	0	0	2	3	2	1	3
S. typhy	3	2	2	1	0	2	2	1	1	2	2	1	1	1	2
S. pararatyph	2	3	1	1	2	3	3	1	0	1	1	2	2	1	0
Shigella dysenteriae	2	1	2	0	0	2	2	1	1	0	1	2	1	2	0
E coli	1	1	2	0	1	3	1	2	1	1	2	3	1	2	0
Enterococci	3	1	2	2	1	2	2	1	2	0	3	1	2	3	1
Fe coliforms	3	2	1	0	0	2	2	1	1	2	3	2	1	0	0
T. coliforms	2	3	0	2	1	2	2	0	0	0	2	3	1	2	1

1.6.2.2. Effect of Cd on the growth of bacteria isolated in the sediment

Table 35: Average number of Cd-tolerant bacteria in the sediment

Bacteria	**Number of positive plates**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	3	2	1	2	1	3	3	2	2	1	2	3	1	0	1
S. typhy	2	2	1	1	0	3	3	1	2	1	2	2	1	1	1
S. pararatyph	3	3	1	2	2	3	3	2	1	1	2	3	2	1	0
Shigella dysenteriae	2	2	3	1	0	2	1	1	0	0	2	1	1	0	0
E coli	2	1	0	1	0	3	2	2	1	0	3	3	2	0	0
Enterococci	3	3	2	1	1	2	1	1	2	2	2	3	2	1	1
Fe coliforms	2	2	3	1	0	3	2	1	0	0	3	1	0	2	0
T. coliforms	3	3	1	0	1	2	2	2	0	0	2	3	1	0	0

1.6.2.3. Effect of Pb on the growth of bacteria isolated in water

Table 36: Average number of Pb-tolerant bacteria in water

Bateri Indic.	**Number of positive plates**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	3	2	2	1	1	2	2	3	3	2	3	3	3	1	2
S. typhy	2	1	3	1	1	2	2	3	2	1	3	2	3	2	1
S. pararatyph	2	1	1	0	2	3	2	2	1	2	3	3	1	2	1
Shigella dysenteriae	2	3	0	2	2	3	1	2	2	0	2	2	1	2	1
E coli	3	3	2	1	2	3	2	1	2	0	2	3	1	0	1
Enterococci	2	2	3	2	2	3	3	2	1	2	3	2	3	0	2
Fe coliforms	3	2	1	1	2	2	1	1	0	2	3	2	1	1	0
T. coliforms	3	3	2	2	1	3	1	2	2	0	3	2	1	2	0

1.6.2.4. Effect of Pb on the growth of bacteria isolated in the sediment

Table 37: Average number of Pb-tolerant bacteria in the sediment

Bateri Indic.	**Number of positive plates (n=3)**														
	Site I					**Site II**					**Site III**				
	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5	0.25	0.5	1	1.25	1.5
Vibrio choler	2	3	3	2	3	3	3	3	1	1	3	2	2	2	1
S. typhy	3	1	3	2	2	3	1	2	2	1	3	1	2	3	2
S. pararatyph	2	3	1	1	2	3	3	1	2	0	3	2	2	1	1
Shigella dysenteriae	3	3	1	2	2	3	2	2	1	0	2	2	1	0	1
E coli	2	1	2	1	1	3	2	3	2	1	2	1	0	1	2
Enterococci	2	2	2	1	3	2	3	1	2	1	2	1	2	1	1
Fe coliforms	2	2	1	2	2	3	2	3	1	1	2	2	1	2	2
T. coliforms	3	3	1	2	2	2	1	2	3	2	2	2	3	2	1